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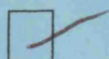
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Photodynamic Therapy for the Treatment of Oral Cancer

Thesis submitted to the University of London
for the degree of MD

By

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2006

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Abstract

Photodynamic therapy (PDT) describes an interaction of a drug, light and oxygen that results in cell killing. The aim of this thesis is to describe the potential clinical applications of the three most commonly used drugs in PDT – aminolaevulinic acid (ALA), Photofrin® and Foscan®. Before it was considered safe to use this therapy on patients, a series of preclinical studies were undertaken to establish the safety of the treatment and to try to understand the likely clinical effects on normal tissues.

Following this, a series of studies was undertaken to look at the biological effect of PDT on normal and neoplastic tissue. These studies showed the treatment to be safe and effective in destroying tissue while allowing healing with preservation of sufficient tissue structure to maintain tissue contour and function.

Next, clinical studies were undertaken on dysplasia, early oral cancer and field change disease using the 3 sensitisers. ALA was found to be useful in the treatment of dysplasia, but has a very superficial effect so is not indicated for treatment of invasive disease. Photofrin® and Foscan® have a much deeper effect and can be used to treat early cancer and superficial field change disease. Both drugs have problems of prolonged sensitivity to light varying from 2 weeks (Foscan®) to 3 months (Photofrin®). Treatment times also vary from 200s (Foscan®) to 1000s (Photofrin®).

The depth of effect limits the use of surface illumination to a maximum of 1cm, however, the use of interstitial therapy, where the light is delivered directly into a neoplastic tissue target allows more advanced cancers to be treated.

Currently, Foscan® is the only drug licensed for head and neck cancer and then only in the advanced or palliative setting where all other options have been exhausted or are not appropriate. While there is no single drug or technique for the treatment of all stages of oral cancer, PDT is now beginning to be used alongside surgery, radiotherapy and chemotherapy.

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Statement of Originality

The preclinical experiments described in chapter 6 were carried out by Mr W Grant and Ms K Fan under the supervision of Prof Bown and myself. The experimental design was developed by Prof Bown, myself and either Mr Grant or Ms Fan. It was felt valuable to include this work as it formed the basis of the safety data required for human studies. The subsequent interpretation and publication of this work was collaborative with the majority of the work provided by Mr Grant and Ms Fan. The majority of data from this chapter has been taken from papers published or in preparation for publication where I am a co-author. The subsequent chapters dealing with PDT treatment of the different stages were carried out by me or supervised by me on my patients for whom I had total responsibility. I have been the principle investigator on all of the ethics applications for these studies.

The Foscan® study in chapter 9 was the output of a writing committee of which I was chairman. The patients treated by me are listed in the chapter. Chapter 8 -Foscan® treatment of dysplasia is exclusively my work as are Chapters 10 and 11 where I have developed these treatments over several years. The interstitial approach (Chapter 11) was developed with input from my radiology colleagues, Dr Rolf Jäger and Dr Joe Brookes.

Some of the data in chapters 6 and 7 appears in part in the higher degrees of Mr Grant and Ms Fan, but the clinical studies represent my work over the last 12 years. A more detailed statement of contributions from others appears in the introduction of Chapters 6 – 10.

Dedication

**To Yvonne, Jonathon and Elisabeth, without whose
forebearance this thesis would never have been
finished**

Acknowledgements

This work has only been possible with help and advice from very many people over several years. My initial interest was stimulated by Prof S G Bown at the National Medical Laser Centre following a chance meeting just after my appointment as Senior Lecturer at UCL. He has been a constant support and sounding board for new ideas and has been instrumental in overcoming some of the complex funding issues associated with research. A series of research fellows has also been essential to help establish safety parameters before we started clinical studies before any PDT drugs were licensed – Will Grant, Kathy Fan, Alex Lou, Jem Prabhakar, Hiroaki Nakanishi, Manfred Suhr and Tamer Theodossy.

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Chapter 1: Oral Cavity Cancer - Epidemiology, Risk Factors, Histopathology and Prognosis

1.1 Scope of this Thesis

Head and neck cancer includes cancers of the oral cavity, larynx, oral- and hypopharynx and salivary glands. The oral cavity includes the lips, buccal mucosa, anterior tongue, floor of mouth, hard and soft palate, upper and lower gingiva. Cancers of the oral cavity (orofacial cancers) comprise 40% of head and neck cancers and this thesis will concentrate on management of these cancers. (ie Lip, Oral cavity ICD 140,141,143-145 – Appendix 2).

Squamous cell carcinomas (SCC) arise from the squamous epithelial cells lining the upper aerodigestive tract. These malignancies, and pre-malignant conditions, will be discussed in this thesis. In general, other carcinomas (such as basal cell carcinoma, adenocarcinoma, adenoid cystic carcinoma and pharynx - ICD 140,146-148 salivary glands ICD 142) are outside the scope of the thesis except in cases of advanced disease where palliative interstitial treatment was used. (Chapter 11).

1.2 Epidemiology

1.2.1 Geographical Distribution

Worldwide oral cavity cancer constitutes 267 000 cases, amounting to 2.7% of all cancers (Parkin et al 2001). While it is difficult to get accurate up to date figures, it is estimated that with accurate reporting, the most recent worldwide figures suggest there is little change. Globocan 2002 estimates are 274,289 (Parkin et al 2005).

The highest incidences of head and neck cancer are found in Singapore and India. In South East Asia and particularly in India, cancer of the oral cavity is the most common cancer, comprising 35% of all cancers in men and 18% of all cancers in women (Shah et al 1999). The high incidence in South East Asia is attributable to the local habit of chewing *paan* (a mixture of tobacco, areca nut,

lime and other substances wrapped in a betel leaf). High rates are also reported for parts of South America, the Bas Rhin region of France (said to be related to Calvados ingestion), Eastern Europe and countries around the West Pacific.

In the US, American Cancer Society data suggests oral cavity cancer is the ninth most common cancer with 27,700 new cases per annum, accounting for 2% of all malignancies, with a death rate of 7,200. In 1997, new cases of cancer of the oral cavity were estimated at 22,000 (Parker et al 1997) and deaths that year were estimated at 6,500. Within the US, there are marked geographical variations due to local habits such as the use of smokeless tobacco in the rural South (Winn et al 1981). Estimates from the American Cancer Society for 2005 are similar to these figures.

In 1990, Black et al (1997), estimated oral cancer (ICD-9 140-149) as the sixth most common cancer in men in the European Union (5.6% of all cancers for men, 1.4% for women **Table 1.1**).

	Cases Per year	% all cancers	Mortality	% mortality (all cancers)
United Kingdom	3655	1.6%	1932	1.2%
UK Males	2295	2%	1201	1.4%
UK Females	1360	1.1%	731	0.9%
European Union	48927	3.6%	19282	2.2%
EU Males	40240	5.6%	15467	3.1%
EU Females	8687	1.4%	3815	1%

Table 1.1: Oral Cancer Incidence (UK and EU, after Black et al 1997)

The incidence amongst the 38 countries in the United Nations defined area of Europe of oral cavity cancer (ICD 140 – 145) was nearly 50,000 cases (Bray et al 2002). The mortality in 1990 was estimated at 19,200. Across the European Union there are wide variations in the incidence, with that of France (approximately 50/100,000 men/year) being almost seven times that for Greece.

Such large geographical variation reflects differences in lifestyle risk factors such as alcohol drinking, tobacco smoking and the kind of tobacco used, along with dietary habits, all of which may have an independent effect on prognosis (Esteve et al 1996, Crosignani et al 1996).

1.2.2 Incidence by Anatomical Site

Cancer of the oral cavity is the most common site of head and neck cancers, comprising 40% of the total. By site of occurrence, the incidence in the US is as follows (Rice & Spiro 1989):

Oral cavity	40%
Larynx	25%
Major salivary glands	17%
Oral/hypopharynx	15%
Remaining head and neck sites	13%

Table 1.2: Cancer Incidence by Site in the Head and Neck

Within the oral cavity, the vast majority of tumours are squamous cell carcinomas of the mucosa, tongue and lip. Most primary squamous cell carcinomas of the oral cavity are located in those areas of the oral mucosa that are exposed to saliva: the lateral border and under-surface of the tongue, and the floor of the mouth (Shah et al 1999).

In descending order, the site distribution of primary SCCs is:

- Tongue
- Floor of mouth
- Cheek
- Gum
- Retromolar trigone
- Lip
- Soft palate
- Hard palate

In the areas of South East Asia and India where there is widespread use of *paan*, there is a correspondingly high incidence of carcinoma of the cheek mucosa, the retromolar trigone and base of the tongue, at the site where the quid is held in the mouth.

1.2.3 Incidence by Gender

In the US, cancers of the oral cavity are far more common in men, affecting 16.7 per 100,000 compared with 2.6 per 100,000 women. In all developed countries, the incidence of head and neck cancer in women is lower than in men (the overall annual age standardised incidence rate in the European Union has been estimated at 26 per 100,000 in men and 3.1 per 100,000 women (Black et al 1997) and geographical variability is less pronounced in women. However, over the last 50 years, the incidence of tongue carcinoma in women has nearly doubled, rising from 15% of all tongue cancers in the 1930s to 40% in the 1980s (Franceschi D et al 1993) probably due to an increase in consumption of tobacco and alcohol.

In the US, the incidence of carcinoma of the upper aerodigestive tract is two to three times higher in men than in women (Blair et al 1994) but the incidence in women continues to rise, probably as a result of increasing numbers of female smokers.

In areas such as India, where chewing and smoking are common among women, the incidence of tongue and other oral cavity cancers for women is greater than, or equal to, that for men.

The incidence also varies between socio-economic and racial groups. While the incidence and mortality of oral cavity cancer in white men has been falling slightly in the past 50 years, there has been a relative increase in black males. Black patients demonstrate inferior survival in all head and neck sites when compared with their white counterparts and the initial stage of disease at presentation tends to be more advanced (Parker et al 1997).

1.2.4 Incidence by Age

The incidence of head and neck cancer increases with age, with around 90% of all cases being diagnosed after the age of 40 (Miller et al 1990). The majority are diagnosed in the sixth and seventh decades of life (Rice and Spiro 1989).

1.3 Risk Factors

Certain patients have a high genetic and familial predisposition to the development of cancer, however, the influence of tobacco and alcohol exposure to the upper aerodigestive tract over-rides any underlying genetic predisposition that may exist.

1.3.1 Tobacco

The most significant risk factor for the development of head and neck cancers is smoking, with 90% of head and neck cancers occurring in smokers. Many studies have confirmed the association between smoking and head and neck cancer (for example, Keller et al 1965, Kahn 1966, Hinds et al 1980). The risk is proportional to the amount smoked (Spitz et al 1988). In parts of the world such as Andhra Pradesh, India, where the practice of reverse smoking is common, there is an extremely high incidence of oral cavity carcinoma (Reddy DG 1957, Reddy CR 1974).

Other forms of tobacco also contribute to the development of oral cancer. Chronic use of *paan* is associated with a high incidence of carcinoma of the cheek mucosa, retromolar trigone and base of the tongue in India (Shah et al 1999, Schonland et al 1969). Smokeless tobacco, which is perceived by some to be a safer alternative to cigarettes, is associated with 50-fold increases in the incidence of gingival and buccal cancer in chronic snuff users in the US (Winn et al 1981, Schottenfeld 1981).

1.3.2 Alcohol

The addition of alcohol increases the risk of developing head and neck cancer (Keller et al 1965, Schottenfeld 1979). Either substance alone increases the risk of development of head and neck cancer (Herity et al 1982), but when tobacco and alcohol are used together, a synergistic effect increases the relative risk for development of cancer many times compared with non-users (Rothman et al 1972 **Table 1.3**, McMichael 1978, Hinds et al 1980). While a non-drinking heavy smoker is between two and a half (Rothman et al 1972) and five (Thompson 1989) times more at risk of developing head and neck cancer than a non-smoker, a heavy smoker who also drinks heavily increases the risk by a factor of 15 (Thompson 1989).

	Cigarettes per day			
Ethanol per day	0	1-20	21-39	40+
0 cm ³	1.0	1.5	1.4	2.4
1-11 cm ³	1.4	1.7	3.2	3.3
12-45 cm ³	1.6	4.4	4.5	8.2
46 cm ³ +	2.3f	4.1	9.6	15.5

Table 1.3: Relative Risk of Squamous Cell Cancer of the Oral Cavity Stratified by Tobacco and Ethanol Consumption

From Rothman et al 1972, computed from a case-controlled study of patients in the Veterans Administration Hospitals

Direct contact of the carcinogenic agents in smoke and alcohol appears to be an important factor in the mechanism of tumour development, hence the weak association between alcohol consumption and cancer of the larynx where contact is minimal (Russo et al 1996). These agents appear to induce field changes in all the mucous membranes with which they come into contact leading to multiple primary cancers (Blitzer 1988).

1.3.3 Genetic Basis

This complex area of research has suggested that a number of genetic events are necessary for cancer to develop. Alterations of tumour suppressor genes on chromosome 9p and 3p are present in oral cancer and precancer (Roz et al 1996, Califano et al 1996). Forastiere et al, in the 2001 review, give an overview of this subject. They postulate that head and neck cancer derives from molecular changes in a common clonal progenitor cell, with inactivation of tumour suppressor genes, (most frequently p16^{INK4A} and p53) or amplification of proto-oncogenes.

Some genetic studies of oral cancer suggest that single cell changes give rise to clonally related transformed damaged cells so that in patients with field change disease with multiple cancers arising within the same field these are often thought to be of a single clonal origin (re: Califano et al 2000 and Bedi 1996). These clonal changes can affect large areas of the mouth and there is data to suggest that tissue most likely to progress to cancer comes from clonal genetic changes (Mao et al 1998).

The use of polymerase chain reaction technology has revolutionised our understanding of carcinogenesis. Studies using extracted DNA from normal, pre-malignant and malignant tissue has allowed the genetic changes that happen with progression towards cancer. The essential events are of allelic losses (chromosomal deletions) which result in tumour suppressor gene inactivation. This requires a two step inactivation process and a point mutation of one allele followed by deletion of the second allele resulting in complete loss of tumour suppressor function. Specific changes are noted in smoking related tumours with 3P21 being among the most frequently damaged chromosomal regions.

1.3.4 Other Factors

Dietary factors do not appear to play a direct role in the development of SCCs of the oral cavity but may have an effect on survival after diagnosis. It is difficult to separate out the role of diet in the aetiology of head and neck cancer as

nutritional deficiency is commonly associated with chronic alcohol consumption (Kissin et al 1973).

Less significant factors may also play a part in the development of head and neck cancer. For example, it has been noted that there was a topographic coincidence between chronic mechanical trauma from dental prostheses and tumour development in 44% of oral cancer cases (Thumfart et al 1978). Some chronic diseases including syphilis, candidiasis, erosive lichen planus, iron deficiency and AIDS have also been shown to be associated with an increased risk of oral cavity cancer (Fry et al 1929, Mackenzie et al 1980, Silverman et al 1974, Watts 1961, Silverman et al 1986). Viral conditions, especially HPV type 16, have been associated with squamous cancer of the head and neck. However, recent PCR work has suggested the importance of this has been exaggerated (Ha et al 2002).

Finally, there are some work-related risk factors where exposure to high levels of carcinogenic substances or sunlight induces cancers of the lip or oral cavity. Another example is the high risk of tongue cancer associated with using radioactive paints to paint watch faces although this is now of historical interest only.

1.4 Histopathology

Nearly 95% of cancers of the oral cavity arise from the surface epithelium and are therefore SCCs, the remainder being minor salivary carcinoma, melanoma, lymphoma and sarcoma (Batsakis 1974).

Microscopically, SCCs are graded according to cellular differentiation as well-differentiated, moderately differentiated or poorly differentiated.

Pre-malignant changes are often visible or present before the development of invasive carcinoma and a wider awareness of these changes would significantly improve detection and diagnosis. Most epithelial pre-malignant changes occur as white (leukoplakia) or red discolouration (erythroplakia) which are usually easily visible on examination. These changes reflect epithelial and sub-epithelial histomorphologic disturbances which include disturbances in the

keratin content of the epithelial layer and changes in the cellular cytoplasm. Further epithelial changes lead to development of hyperkeratosis, parakeratosis, dyskeratosis, carcinoma *in situ* (ie confined to the epithelium) and eventually invasive carcinoma extending through the basement membrane to the underlying tissue. These changes are not observed in all patients.

The risk of leukoplakia developing into invasive carcinoma has been estimated at approximately 4% to 6% (Pindborg et al 1968, Silverman et al 1984, Waldron et al 1975), while the risk of erythroplakia developing into invasive carcinoma is as high as 30% (Mashberg et al 1973). These terms have largely been abandoned and white or red patches are now graded in histological terms. Mild dysplasia is associated with a low risk of transformation (<5%) and severe dysplasia with a much higher lifetime risk of about 50%.

A varying degree of keratinization is usually present on the surface of a primary tumour, the extent of which is a reflection of the degree of differentiation of the underlying malignant process. Highly keratinized tumours are usually well differentiated and considered less likely to metastasise than poorly differentiated SCCs which show very little or no keratinization. Local extension by spread to adjacent structures may lead to invasion of the underlying soft tissues and muscles, bone or neurovascular structures. The spread of the tumour usually occurs by local invasion through lymphatics to regional lymph nodes or less commonly via bloodstream dissemination to distant sites.

1.5 Prognosis

In general, age-adjusted cancer death rates for patients with cancer of the head and neck have remained unchanged over the past 30 years (Silverberg et al 1990). More recent data from Cancer Research UK suggests the situation has not changed significantly since this time (CRUK 2005).

Parker et al (1997 **Table 1.4**) report that overall five-year relative survival rates for cancers of the oral cavity and pharynx have remained unchanged at around 55% since 1974 for white people and have in fact declined slightly for blacks (36% down to 33%). Reported survival rates for specific cancers suggest that

there has been little improvement for over 40 years (Martin et al 1940, Harrold 1971, Martin et al 1935).

Oral cavity and pharynx	1974[§]	1997[¥]
- all stages	55%	55%
- localised	78%	82%
- regional involvement	43%	44%
- distant metastases	20%	20%

Table 1.4: Representative Five-Year Relative Survival Rates for Cancers of the Oral Cavity and Pharynx

§ Boring et al 1994; ¥ Parker et al 1997

Small improvements in survival rate have been claimed only in a few highly specialised treatment centres based on experience gained from a large number of cases (Shah et al 1999, Miller et al 1990).

Second primaries are the major threat to long-term survival after therapy of early stage head and neck squamous cell carcinomas. The high incidence of second primaries in this area probably arises from the same exposure to carcinogens that were responsible for the initial primary.

Several studies have demonstrated that the rate of development of second primary tumours in these patients is 3 to 4% per year, with 10.6% of patients developing a new primary over an average 3.4 year follow up (Day and Blot 1992, Jovanovic et al 1994). This feature of oral cavity cancer is often referred to as "field cancerisation" where an entire region of tissue is exposed to carcinogens, thereby increasing the risk of that tissue developing multiple independent pre-malignant and malignant areas (Slaughter et al (1953), Lippman and Hong 1989 and 1992, Strong et al 1984).

Most patients (70% to 80%) present late in their disease course with advanced loco-regional tumour. The majority of patients with squamous cell carcinoma of the oral cavity die of local and cervical node recurrence and progression, while only around 10% die of distant metastases. (Vokes et al 1993, Dimery et al

1993, Ildstad et al 1989). Clearly, improved local control strategies are the key to improved survival.

1.5.1 Site of Tumour

The site of the primary tumour has prognostic importance although this may partially reflect differences in the early recognition of symptoms and ease of assessment and treatment once identified. Early stage cancers of the oral cavity have a good prognosis with fairly simple treatment: five-year survival rates range from 75% to 85%. With advanced cancer the five-year survival falls to about 35% although many deaths are due to intercurrent disease.

The EURO CARE 2 study confirmed that five-year survival rates were highest for lip cancer, but low for cancers of the tongue and other parts of the oral cavity. Five-year relative survival of male patients with cancer of the tongue, mouth and pharynx was 34% (Sant 1999).

Tobias (1994) in his review of the data suggests that survival according to site decreases in the following order:

- Lip
- Larynx
- Oropharynx
- Nasal sinus
- Oral cavity
- Nasopharynx
- Hypopharynx

In advanced disease, the following survival rates are to be expected:

Cancer site	Males	Females
Pharynx - oropharynx	26.3	36.4
- nasopharynx	31.1	39.8
- hypopharynx	15.1	18.5
Oral cavity - alveolus	40.7	58.3
- floor of mouth	39.0	52.3

Table 1.5: Five-Year Percentage Survival Rates in Patients with Advanced Head and Neck Cancer (initial treatment with combined chemotherapy and radiation with surgery for resistant or recurrent disease)

Tobias 1994 after Cancer Research Campaign facts on Cancer Factsheet No 9

1.5.2 Stage of Disease

Although location of the tumour is a significant independent factor influencing survival, clinical stage at initial diagnosis is the single most important prognostic factor (see Appendix 1 for an explanation of the staging system). This is the case for all tumour sites (Ildstad et al 1989). Patients with more advanced disease, represented by a larger primary tumour or more nodal involvement have less favourable survival prospects, thus confirming the importance of early diagnosis and treatment of oral malignancies.

Five-year relative survival rates are strongly linked to stage at diagnosis: patients diagnosed while the disease is still localised are four times more likely to survive than those presenting with distant spread of disease (Parker et al 1997 **Table 1.6**).

Stage	Tongue	Floor of mouth
I	65	78
II	53	65
III	29	43
IV	20	18

Table 1.6: Overall Average Five-Year Survival for Patients with Squamous Cell Carcinoma Tumours (Parker et al 1997)

Each component of T, N and M is independently predictive. Nodal status in particular is highly predictive (South East Cooperative Oncology Group 1986) with the probability of spread to lymph nodes being highly dependent on the primary tumour site. Nasopharyngeal carcinomas, supraglottic larynx, floor of mouth, oropharyngeal and hypopharyngeal sites have very high rates of spread (Tobias 1994).

The location of the primary tumour is likely to dictate which nodes are involved but the sub-mandibular is the most common (level I) followed by upper, middle and lower cervical nodes (levels II - IV). The more node groups that are involved, the more serious is the prognosis for the patient, as this indicates more extensive spread of disease.

1.5.3 Other Factors

Geographical differences in survival between Western European countries are probably due to a difference in the case-mix of anatomical sub-sites, whereas the difference in survival between Eastern and Western European countries is more likely to be due to late diagnosis and referral or poor access to adequate treatment in the former (Berrino et al 1998).

Socio-economic background of patients with squamous cell head and neck cancer plays a significant role in the success of disease management. Limited access to medical care often results in patients presenting with advanced disease.

Patient age at time of initial diagnosis and treatment has a significant influence on survival independently of tumour stage or location.

1.5.4 Second Primary Tumours

The co-existence of pre-malignant lesions such as leukoplakia and erythroplakia with an existing primary lesion greatly increases the chance of developing a second primary cancer (Sarasin 1933; Wilkins and Vogler 1957, Moertal and Foss 1958, Shibuya et al 1986).

Leukoplakia, a term describing a white patch or plaque that cannot be characterised clinically or pathologically as any other disease entity (Kramer et al 1978) has a propensity for malignant transformation. The rate of malignant transformation in leukoplakia is approximately 4% to 6% (Pindborg et al 1968, Silverman et al 1984, Waldron et al 1975). Histological grading of leukoplakia gives a different picture, with malignant transformation of oral epithelial dysplasia and leukoplakia with severe dysplasia quoted as 14% and 43%, respectively (Axell 1987, Lumerman et al 1995, Maerker and Burkhardt 1978). The incidence of multiple oral carcinomas in patients with primary tongue carcinomas associated with leukoplakia has been found to be five times greater than that of subjects without leukoplakia (Shibuya et al 1986) and has been variously reported as being from 7% to 30% (De Vries 1990).

The presence of an erythematous component, erythroplakia, or erythroleukoplakia increases almost four-fold the risk of malignant transformation (Silverman et al 1984).

The presence of more than one primary carcinoma has a profound influence on survival, and therefore management. Cohn and Peppard (1980) found that 75% of patients who developed second primary tumours in the upper aerodigestive tract died within 12 months of the second diagnosis. Carr and Langdon (1989) found that the average survival of patients with index oral cancers who developed second primaries was 7 months. Two-thirds of this series died less than 6 months after diagnosis of the second tumour. Gluckman and Crissman

(1983) found that overall five-year survival after diagnosis of a second primary was 22.3% (compared with 34% for a control group with single lesions).

Close follow up screening of all patients with a diagnosis of upper aerodigestive tract carcinoma is necessary to facilitate early detection of a second primary and recurrent disease.

1.6 Summary

From the available evidence it is clear that the aetiology of oral cancer is complex, with many factors evolving over time. However, chronic exposure of the oral cavity to alcohol and tobacco smoke especially when coupled with habits such as *paan* chewing result in widespread genetic alterations that lead to dysplasia and invasive cancer. Once this train of events has been set in motion, patients who develop oral cancer once have sufficient genetic damage to remain at risk of further metachronous tumours for the rest of their lives.

Chapter 2: Diagnosis and Management of Oral Cancer

2.1 Diagnosis

2.1.1 Clinical Presentation

Cancer of the oral cavity may present in many ways and any of the following signs are regarded with suspicion: ulceration which fails to heal, induration of a mucosal lesion, fungation or growth, fixation of the mucosa, failure to heal of a wound or extraction, white or red patches of the mucosa, pain or dysphagia with no apparent cause. Epithelial dysplasia indicates an increased risk of malignant change which may be prevented with early intervention.

Tumours usually appear as whitish plaques or ulcerations. They may be:

- Ulcerative: usually accompanied by irregular edge and induration of the underlying soft tissue
- Exophytic: may present either as a cauliflower-like irregular growth, or a flat pinkish-white proliferative lesion. SCCs with excessive keratin production and verrucous carcinomas appear as white lesions with varying degrees of keratin debris on the surface
- Endophytic: small surface component with substantial soft-tissue induration beneath the surface
- Superficial proliferative lesions

White lesions may be confused with mucositis, candidiasis, lichen planus or simple tissue irritation. Those that persist despite removal of local irritating factors should undergo biopsy.

Exophytic carcinomas usually have clear borders and also have a lower rate of lymph node metastasis. These lesions are suitable for surgery or interstitial radiation as the borders are well-defined and the chance of missing the margins is low. Overall, they tend to respond better to treatment of any kind.

The poorly differentiated tumours tend to be more infiltrative, with fingers of tumour cells extending far beyond the visible borders. This growth pattern is associated with a higher rate of regional lymph node metastasis and is usually more difficult to treat with surgery.

Dysphagia, glossodynia, otalgia, hoarseness, pain, weight loss and unexplained neck mass are very common presenting symptoms requiring further investigation.

2.1.2 Vital Staining

A number of vital staining techniques have been investigated such as toluidine blue and lugols iodine. The only commonly used technique is a modified toluidine blue application that is commercially available as Orascreen. This can increase diagnostic yield especially for the inexperienced clinician. (Mashberg et al 1989).

2.1.3 Optical Interrogation

Normal tissue has certain physical properties such as fluorescent impedance and light scattering that are different to those seen in abnormal tissue, either cancerous or pre-cancerous. These changes can be exploited in a non-invasive fashion using optical interrogation. Currently, Raman spectroscopy, auto- and enhanced fluorescence and elastic light scattering are all being investigated. The techniques tend to be inexpensive but as yet are not fully validated.

2.1.4 Genetic Analysis

There has been a great deal of interest in genetic changes (aberration) in oral squamous cell carcinoma. It is clear that series of molecular genetic events coupled with a reduced ability to repair or destroy dysfunctional cells is a precursor to the development of cancer. Identification of these changes (biomarkers) may help improve surveillance and identify high risk pre-malignant lesions.

2.1.5 Imaging Techniques

Tumours of the head and neck can be fairly readily inspected and palpated, but there are occasions where imaging is appropriate and necessary, for instance, to assess the extent of bone invasion by a tumour of the jaw. Magnetic Resonance Imaging (MRI), Computerised Tomography (CT) and ultrasound scans (US) are important tools in the assessment of advanced tumours, particularly those in non-surface sites such as the base of the skull.

In evaluation of the nodal neck disease the issues are not so clear. CT and MRI are routinely used in disease staging. The management of the No neck is contentious and in the best hands ultrasound guided fine needle aspiration cytology increases sensitivity and specificity. Positron Emission Tomography is now in routine use in major centres around Europe and the United States. It images glucose avid tissues (the brain and tumours have increased glucose turnover) and at the same time localises this tissue to a CT scan to facilitate anatomical interpretation. This is particularly useful for identification of second primaries and metastases. It is also thought useful for follow up surveillance. Sentinel node studies have an established role in tumour staging of malignant melanoma and to some extent oral cancer. Both are the subject of clinical trials but are outwith the scope of this thesis.

2.2 Treatment Options for Oral Cancer

Clinically these tumours pose exceptional problems in management due to the poor prognosis in advanced cases, the inter-current medical problems frequently experienced by patients, and the adverse effects of treatment on oral and pharyngeal function. The proximity to vital structures in the head and neck region makes treatment difficult and often severely deforming.

Tumours in the oral cavity are often referred to as a single entity but are in fact a heterogeneous collection, with the natural history, prognosis and preferred therapy heavily dependent on the site of the original tumour. The goals of treatment are:

- To cure the cancer
- To preserve or restore form and function
- To minimise sequelae of treatment
- To prevent second primary tumours

Unfortunately, with existing treatments, the attainment of some of these goals is usually only achieved at the expense of others.

Surgical resection and radiotherapy, either singly or combined, are currently the main treatments for oral cancer. For small primary cancers (stages I and II, without regional spread), either technique on its own can provide good local control and long-term survival but often at the cost of functional or cosmetic impairment in the case of surgery, or short- and long-term side effects in the case of radiotherapy.

The initial choice of treatment is influenced by:

- site, size, location of tumour, proximity to bone and pathology
- status of cervical lymph nodes
- previous treatment
- patient's age, general medical condition, occupation, tolerance to treatment, anticipated compliance with treatment, lifestyle and socio-economic status

A patient with, for example, an early tumour of the larynx may be best treated with radiation initially as surgical treatment would almost certainly result in significant disruption to normal function. Multiple simultaneous primary lesions can be more easily treated with radiation than surgery. However, small lesions of the oral cavity - such as those on the lip, lower gum and retrimolar trigone, tip of the tongue, epiglottis, - can be treated effectively with surgery, with little disruption to function or cosmesis, thus avoiding the long-term adverse effects associated with radiation.

The oral cavity has several subgroups of patients who pose particularly difficult therapeutic challenges.

Dysplastic lesions of the oral mucosa may affect multiple sites and have diffuse margins, however, there are no widely accepted criteria for defining the presence and degree of dysplasia. It is also unclear which lesions require treatment, nor how aggressively such lesions should be treated (Speight et al 1993). Malignant transformation rates vary from 4% to 6% (Pindborg et al 1968, Silverman et al 1984, Waldron et al 1975), while the risk of erythroplakia developing into invasive carcinoma is as high as 30% (Mashberg et al 1973). Leukoplakia may occur in conjunction with one or more primary tumours, – a condition first described by Slaughter et al (1953) as field carcinogenesis or field cancerisation. Several studies have demonstrated that the rate of development of second primary tumours in these patients is 3 to 4% per year (Day and Blot 1992, Jovanovic et al 1994). Many such patients exhibit a field change effect, so it may be necessary to treat large areas. Clearly a more helpful way of viewing this phenomenon is based on the genetic changes found in all these conditions (see 1.3.3).

In these circumstances, it may be difficult to treat all affected areas at any one time, so re-treatment may be necessary. Consequently, a modality that can be applied to large areas of the oral cavity that has little cumulative toxicity would be valuable.

All other factors being equal, isolated tumours in young patients may best be treated with an operation, to avoid the risk of long-term radiotherapy complications, while an elderly or frail patient may well be referred for radiotherapy even for lesions that could easily be treated by surgery.

2.2.1 Surgery

Surgery allows for more accurate tumour staging and determination of the pattern of spread of invasion and, given the high incidence of subsequent primaries in the head and neck, there is an advantage in withholding radiotherapy for subsequent use if required. However, if regional nodal involvement is suspected, then early radiotherapy of the nodes at risk is advised.

Conventional ablative surgery of the oral cavity involves tissue loss, scarring and the risk of damage to adjacent or underlying structures, as well as an overall 30 day operative mortality rate of around 1% to 2%. Early complications of surgery include haemorrhage, aspiration pneumonia and wound breakdown. Delayed complications include tissue loss – particularly innervated tissue and contracture which affects function and appearance: however skilled the surgeon may be, operations for intra-oral carcinoma produce deformity and loss of function (Marchetta 1976).

Sophisticated reconstructive procedures have been developed and are often cited as evidence that surgery to the head and neck region can be non-mutilating, but these are usually only available in highly specialised centres where they reflect the concentration of excellence associated with such institutions. Even in centres of excellence, patients who undergo glossectomy or composite resection will have significant residual cosmetic and functional debilities.

For many patients, surgery is simply not an option, because of generally poor health, or perhaps because of previous surgery to the head and neck region.

2.2.2 Radiotherapy

Radiation therapy is effective in the treatment of loco-regional disease. For early lesions, it gives results comparable to those achieved by surgery. SCCs of the head and neck are moderately radio-responsive, meaning that fairly large dosages of radiation are required to achieve good tumour control but these dosages are generally within the tolerance of the various structures of the head and neck. Many different radiation treatment schedules are in use and under investigation. In the US, a regimen of 180 to 200 cGy once a day for five days over six weeks has become standard for most patients (Eisbruch A et al 1999).

It is generally accepted that radiotherapy preserves function and cosmesis better than surgery but the treatment course itself is usually prolonged (five to seven weeks) and there are many associated short- and long-term unwanted effects.

The primary target of radiation in the cell is DNA and most radiation-induced cell death results from radiation-induced DNA damage. This interferes with the reproductive capability of the cell which is usually manifested after several cell divisions. The effect therefore depends on the number of cells actively proliferating and the length of the reproductive cycle. Acute radiotherapy effects occur in tissues with high cell turnover: skin, bone marrow, gastrointestinal mucosa and hair follicles.

Short-term effects include skin desquamation, mucositis and aspiration. Radiation kills the stem cells in the basal layer, and several weeks later the cells in the superficial layers are not replaced when they are lost through normal physiological processes. This denudes the epithelium, giving rise to mucositis which greatly inhibits a patient's ability to swallow. The daily treatment schedule, in which the delivery of radiation is delayed, evolved to allow regeneration of normal tissue in an attempt to minimise this effect.

The major limiting factor to treatment is the total radiation dose, which can cause long-term unwanted effects on normal tissue. Long-term complications include dry mouth, muscle and subcutaneous fibrosis, soft-tissue and bone necrosis, spinal cord damage, blindness, cranial neuropathy, hypopituitarism and hypothyroidism (Berger et al 1977, Decroix et al 1981, Million and Cassisi 1994, Samaan 1975). Eisbruch A and Dawson (1999) estimate that a total of 65 to 79GY delivered in fractionated doses would lead to a 5% risk, over 5 years, of developing osteoradionecrosis (ORN).

Salivary gland function and taste perception are altered by radiation (Mossman 1983, Mossman et al 1979 & 1981). Decrease in saliva (xerostomia) and changes to its chemical composition cause alterations in the micro-organisms inhabiting the mouth, which in turn can cause a marked increase in caries. To reduce this problem and the potential for osteoradionecrosis, prophylactic dental treatment, including extractions, is usually carried out before treatment rather than waiting until problems occur in a heavily irradiated field.

Modern radiation techniques have diminished, but have not eliminated, these problems.

Concern has also frequently been expressed about the possibility of radiotherapy converting pre-malignant disease into anaplastic and frankly invasive carcinoma, and the long-term effect of radiation on the regional field mucosa remains unclear. Normal oral mucosa exposed to ionising radiation during the treatment of orofacial tumours displays abnormal DNA profiles, which although returning to normal within six weeks, retains the potential for latent radiation damage (Ogden et al 1989). Children and adolescents exposed to irradiation show a predisposition to neoplasia in the head and neck (Martin et al 1970, Southwick 1977, De Groot et al 1983). Robinson et al (1991) report decreased mean survival times for patients with second primary head and neck cancers where index tumours received radiotherapy over patients who had surgery alone. For simultaneous primary cancers surgical resection may be feasible if two or more primary lesions are in close geographical proximity, but may require extensive reconstruction with unacceptable loss of function and cosmesis if occurring at different sites.

2.2.3 Combined Therapy

Patients with moderately advanced lesions will usually benefit from combined therapy given at the outset: loco-regional control rates with single-modality therapy are much less satisfactory than combined therapy in stage III and IV lesions.

The aims of pre-operative radiotherapy are to treat microscopic disease outside the resection field and to shrink the tumour before surgery. A dosage of 50 Gy over 5 to 5½ weeks is usually given (Fletcher 1984).

Used post-operatively, radiotherapy can be tailored more accurately based on the surgical and pathological findings of the initial operation, and a greater knowledge of the tumour spread. However, a larger treatment volume may be required to cover surgical dissections and scars and there will inevitably be a delay to the start of radiotherapy in the post-operative recovery phase. When radiotherapy is post-operative, the site of the tumour has a disrupted blood supply due to the surgery, and higher doses will be required because of the

increased likelihood that tumour cells will be hypoxic and therefore less radio-sensitive. It is therefore usual to give doses of 55 to 60 Gy post-operatively.

Available data suggests that there is little difference in loco-regional control or survival between pre-operative and post-operative radiotherapy but post-operative radiotherapy is the preferred option as operative morbidity is generally lower (Million and Cassisi 1994).

2.2.4 Salvage Procedures

If surgery or radiotherapy fail to control the primary tumour, salvage procedures are often compromised: surgery at the same site is often difficult, sometimes impossible, and repeated radiotherapy is not an option if the patient has already received the maximum allowable exposure of radiation (Million and Cassisi 1994).

The time required for late radiation complications to become clinically apparent ranges from 6 months to 2 years. As many re-irradiated patients do not survive that long, some studies on re-irradiation report an artificially low rate of complications. Stevens et al (1994) recommend re-irradiation only for patients showing minimum clinical evidence of late normal-tissue effects following the first course of radiation. The 5-year actuarial survival for patients selected and treated in this way was 37% for those with new tumours and 17% for those with recurrences. The total radiotherapy dose delivered to the volume of overlap ranged from 69 to 180Gy and severe adverse effects were seen in 9% of patients, including carotid artery rupture, brain necrosis, cranial nerve paralysis and narcotic overdose resulting from the need for strong pain relief.

Surgery can be carried out at previously irradiated sites and vice versa, as in the treatment of more advanced disease where surgery and radiotherapy are often combined, but neither salvage procedure is without difficulty. If irradiation is unsuccessful, the cancer almost always recurs in the centre of the original lesion and a rescue operation may result in a much more severe functional or cosmetic loss than if it had been performed initially. Also because radiotherapy produces an irreversible endarteritis obliterans, tissue oxygenation is

permanently impaired. This diminishes tissue healing, hence surgical complication rates are generally increased following radiation. If surgery is performed as the primary treatment, there is concern about tissue oxygenation as a result of scar tissue formation. Radiotherapy response rates fall in areas of reduced oxygenation, so generally radiotherapists prefer to treat primarily and reserve surgery for salvage. Surgical recurrences usually develop at the margins of the resection where it is difficult to distinguish the normal surgical scar from recurrent disease. Diagnosis of recurrence is therefore often delayed and response of the tumour to radiation is poor. Salvage of surgical resection by radiotherapy is less effective than surgical salvage of radiotherapy failure (Wolf et al 1993).

Overall, salvage surgery or radiotherapy after initial definitive treatment has a very low success rate (Zieske et al 1986).

2.2.5 Chemotherapy

There is little evidence to support the role of chemotherapy in the management of locally advanced head and neck cancer. Chemotherapy has been shown to produce a high rate of tumour response, with rapid shrinkage when used before radiation or surgery. However, there has been little overall improvement in survival, probably because of the toxicity associated with chemotherapeutic agents.

Two recent reviews confirm that the role of chemotherapy is as an adjunctive to other treatments in advanced disease but has no role in early disease.

Adelstein et al (1996) report that many chemotherapeutic agents demonstrate excellent (anti)neoplastic activity yet have a minimal impact on survival in relapsed patients with median survivals ranging between five and seven months.

El-Sayed and Nelson (1996) reviewed prospective and randomised chemotherapy trials in the management of squamous cell carcinoma of the head and neck region. They confirmed that toxicity and morbidity were significantly increased (relative proportion 2.17) for a survival gain of 11%.

A more recent review (Pignon et al 2000) concludes that the impact on survival is too small to recommend the routine use of chemotherapy, particularly in early disease.

2.2.6 Combined Chemoradiation

Recently, there has been a move towards organ preserving therapies. This approach has been pioneered by Robbins in the USA and Lefebvre in Europe. Most studies have focused on laryngeal cancer, but advanced oral cavity cancer has also been treated. (Kumar and Robbins 2001, Lefebvre JL 2005).

2.3 Treatment Outcomes

Representative two year local control rates for squamous cell carcinoma of various sites of the oral cavity treated by radiotherapy with surgical salvage were reviewed by Laramore (1989). Percentages of each group going on to surgery and causes of death in patients with local control are not specified.

Tumour stage		Local control (%)	Survival (%)
Oral cavity -	T1	80-90	75-80
	T2	60-85	40-60
	T3	30-50	20-30
Floor of mouth -	T1	75-85	70-85
	T2	60-80	50-60
	T3	30-50	15-40
Base of tongue -	T1	80-95	65-85
	T2	60-75	40-55
	T3	40-65	15-20
Soft palate -	T1	90-100	90-95
	T2	75-85	65-75
	T3	60-70	30-40

Table 2.1: Representative two year local control rates and survival for SCC of common oral cavity sites. Note: Includes surgical salvage of radiation failures. After Laramore (1989)

2.3.1 Lip

Lip cancers of less than 2cm in size can be successfully treated with either surgery or radiotherapy with cure rates of about 90% for stage I tumours (Bailey 1977), while still providing the patient with a lip that is competent for speech and chewing and without severely compromising appearance. Larger lesions are best treated with surgical resection and reconstruction (Jesse 1967) to eliminate the risk of osteoradionecrosis of the mandible. Lesions with extensive infiltration, bone involvement or lymphatic metastases are usually managed with combined surgery and radiation.

2.3.2 Oral Tongue

These account for around 25% of oral cavity SCCs. Almost all tumours of the tongue arise in the anterior two-thirds of the tongue on the lateral and ventral surfaces. Around 30-40% of patients present with neck nodes (Decroix et al 1981, Spiro 1985) possibly due to the plentiful vascular and lymphatic supply to the tongue. Surgery and radiotherapy both produce similar local control rates for early tumours (T₁ and T₂).

For Stage I cancers, surgery can control the tumour with good preservation of function. However, for Stage II lesions, although hemiglossectomy achieves good control, it results in significant functional morbidity, seriously affecting speech and deglutition. Interstitial brachytherapy is therefore often selected to reduce the effect on function. To achieve the same control rates as surgery, brachytherapy has to be combined with external beam radiotherapy.

Complications of radiotherapy to the tongue include soft-tissue necrosis which can affect nearly one-quarter of patients and mandibular necrosis which may affect around 13% of patients (Decroix et al 1981).

If the tumour has invaded bone, radiation therapy is less effective, requiring doses that would increase the risk of osteoradionecrosis. Complication rates for surgical salvage of failed interstitial implants are extremely high, particularly from fistulisation, exposed bone and failure of reconstruction efforts (Wolf et al 1993).

2.3.3 Floor of Mouth

The floor of mouth consists of the sulcus lying between the oral tongue and the lower gum or gingiva. Tumours in this region are usually squamous cell carcinomas, with an occasional minor salivary gland tumour being encountered. Unlike early tongue tumours, early floor of mouth tumours frequently involve the adjacent mandible, in which case surgery is the preferred primary treatment, necessitating some bone removal in order to ensure an adequate margin. Reconstruction of the mucosal defect may require local or free flaps and replacement bone segments where bone has had to be removed. Radiotherapy to small floor of mouth cancers usually involves external radiation and brachytherapy. As thirty to forty per cent of patients with floor of mouth tumours have nodal metastases in the neck, bilateral elective neck dissections or radiation to the neck are commonly carried out (DiTroia 1972).

The outcome for early floor of mouth tumours is similar whether they are treated with surgery or radiotherapy (Guillamandequi et al 1980, Nakissa et al 1978) but the preferred option is surgery in order to reserve radiation for salvage therapy (Chu et al 1978, Shaha et al 1984).

2.3.4 Base of Tongue

Tumours of the base of tongue typically present late, and usually have a poor prognosis compared with oral tongue tumours. Although surgery and radiotherapy are equally effective for early base of tongue tumours, surgery is technically difficult because of the problem of exposure and invariably results in significant morbidity. Resections of the base of tongue disturb the swallowing mechanism and often speech. For these reasons primary radiation therapy, is favoured by most institutions for these tumours. This also has the advantage of treating associated nodal metastases, which are seen in about 68% of base of tongue tumours (Gibbs et al 2003).

2.3.5 Gingiva and Buccal Mucosa

These occur most commonly (80%) in the lower gingiva posterior to the premolar teeth. Overall, regional metastases occur in 30% of gingival cancers (Byers et al 1981) and 50% of buccal cancers (Bloom et al 1980). Small, superficial cancers of both sites can be treated with either surgery or radiotherapy with good preservation of function. Radiation therapy has the advantage of including the draining lymphatics in the treatment field but increases the risk of post-treatment fibrosis and trismus. Overall survival rates have improved over recent years as surgical management has replaced radiation therapy as primary treatment in the UK though not in parts of Europe and the USA.

2.4 Summary

While great advances have been made in surgery, radiotherapy and chemotherapy, all have drawbacks. There is clearly a need for a therapy that will achieve at least the same control rates as surgery and radiotherapy, with reduced morbidity, functional impairment and disfigurement, with the option for re-treatment at the same site, and which will not compromise surgery, radiotherapy or chemotherapy should they subsequently be necessary.

Chapter 3: Development of Photodynamic Therapy

3.1 Introduction

Photodynamic therapy has only become a viable treatment for cancer in the last two decades, largely through the efforts of German, French, Dutch and English workers, but the treatment of various conditions with light has a very long history.

One of the most easily understood and appreciated manifestations of the photodynamic effect is the photosynthetic reaction which takes place in all green plants, where natural daylight interacts with chlorophyll to absorb carbon dioxide and produce oxygen. At the same time, singlet oxygen is formed which would be immediately destructive if it were not quenched by orange and yellow pigments in plants.

The production of singlet oxygen is the fundamental action upon which all therapeutic applications of photodynamic therapy are based. In the clinical setting, a photosensitising dye is administered which is subsequently activated by light - usually a laser - the beam of which is directed at the area to be treated. When the sensitizer absorbs light of the appropriate wavelength it is converted from a stable state to the excited singlet state and a longer-lived triplet state. The triplet state interacts with molecular oxygen to produce singlet oxygen which is the highly cytotoxic species responsible for the therapeutic effect of photodynamic therapy.

3.2 A Brief History of Photodynamic Therapy

3.2.1 The Use of Light in Medicine

Nearly 3,000 years ago, the Greeks advocated a practice called heliotherapy in which the body was exposed to sunlight for unspecified beneficial purposes. Without being clear about the mechanisms, Herodotus, in the 2nd century stressed the value of exposure to the sun for "the restoration of health" (Daniell et al 1991). There is evidence that the Egyptians, Chinese and Indians used

light as a therapeutic agent in the treatment of diseases including vitiligo, rickets, psoriasis, skin cancer and some psychoses (Spikes 1985, Epstein 1990). In 1815, it was reported that sunlight was effective therapy for rickets, although the role of vitamin D was not understood until much later. Cauvin, a French physician, stated that "sunlight is a curative agent for scrofula, rickets, scurvy, rheumatism, paralysis, swellings and dropsy and muscle weakness" (Cauvin 1815).

In the late-nineteenth century, a Danish physician, Niels Finsen, was responsible for establishing phototherapy as an area for serious scientific study. He reported in 1893 that treatment of smallpox lesions with red light could prevent suppuration and later established that it was the ultra-violet rays in sunlight that were responsible for their bactericidal properties. This led him to develop a method of treatment for lupus vulgaris using carbon arc phototherapy for which he was awarded the Nobel Prize in 1903 (Finsen 1901).

Princess Alexandra, who was to become the wife of Edward VII, brought her compatriot's technique to England and encouraged doctors in the London Hospital to use phototherapy. Phototherapy is still widely used today to treat neonatal jaundice.

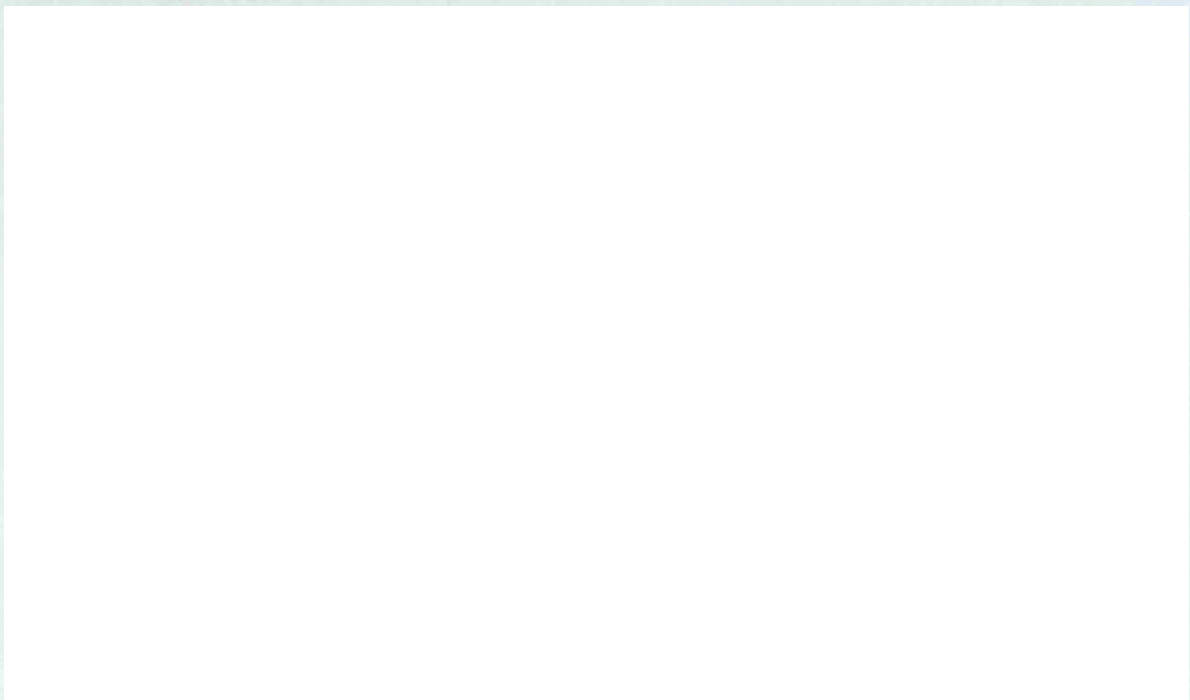


Fig 3.1: Finsen lamp treatment clinic early C20

3.2.2 The Use of Sensitisers

Photochemotherapy, where exogenous sensitisers are used to absorb light, also has a long history, extending as far back as 1400 BC. There are descriptions of treatments in the Atharva-veda which show that Hindus gave oral administrations of extracts of *Psoralea corylifolia* followed by exposure to sunlight, for the treatment of vitiligo. It is now known that these extracts contain furocoumarins which are activated by light (FitzPatrick et al 1959).

In the 12th century AD, the Egyptians obtained psoralens from another plant, the *Anmi majus* which grows by the Nile and used it in the treatment of leucoderma. Much later in 1834, Kalbrunner isolated the active chemical bergapten (5-methoxypsoralen) from bergamot oil but there is no evidence that he used it in the same way as the Indians or Egyptians. It was not until 1974 that psoralen dyes activated by ultraviolet A radiation (PUVA) were found to be effective in the treatment of psoriasis (Harber and Bickers 1981).

The first clear demonstration of the photodynamic effect was made by Raab (1900) in the treatment of paramecia with acridine and light. His experiments led him to conclude that acridine could only kill the paramecia in the presence of light, but light could not kill the paramecia in the absence of acridine (Raab 1900; von Tappeiner et al 1900). Development of the topic by von Tappeiner and other workers led to a recognition that oxygen is the third essential component of the photodynamic effect (von Tappeiner et al 1904) and in 1907, von Tappeiner first employed the term "photodynamic therapy" to describe the process of oxygen-dependent photosensitisation (von Tappeiner et al 1907).

Von Tappeiner was also the first person to attempt phototherapy of tumours in 1904, when he used a topical application of 5% eosin solution - in some cases supplemented by intra-tumour injection - to treat lip cancers.



Fig 3.2: Eosin PDT for lip cancer 1905 (after Von Tappeiner)

3.3 Haematoporphyrin and it's Derivatives

Haematoporphyrin was first made in 1841, by Scherer who added concentrated sulphuric acid to dried blood and washed the precipitate free of iron (Scherer 1841). In 1867, the spectrum and fluorescence characteristics of this substance were described by Thudichum (Thudichum 1867). Hoppe-Seyler first described the purple substance found in iron-free haematin as haematoporphyrin, deriving the name from the Greek 'porphuros' which means red-purple (Hoppe-Seyler 1871).

A number of animal studies were carried out in the early 20th century to establish the biological properties of haematoporphyrin (Hausmann 1908, Hausmann 1911, Pfeiffer 1911) and the first experiment in humans came in 1913 when a German scientist, Friedrich Meyer-Betz injected himself with the substance (Meyer-Betz 1913). After deliberate light exposure he noticed that there was severe pain and swelling in the light-exposed areas and he had to avoid light for over two months.



Fig 3.3: Meyer-Betz pre & post light exposure 1913

Over the next three decades Meyer-Betz and a German chemist Fischer, investigated various porphyrin structures for their phototoxicity, while other scientists began the first experiments of photodynamic therapy in tumour tissue.

In 1924 in Lyon, Policard observed that there was selective localisation of porphyrin to tumour tissue (Policard 1924) and this was confirmed in 1942 by Auler and Banzer, working in Berlin, who investigated the uptake of haematoporphyrin by tumour tissue in experimental tumours and found higher levels than in surrounding tissues (Auler et al 1942).

After the Second World War, Schwartz and colleagues (1955) found haematoporphyrin to be a variable mixture of numerous different porphyrins, the most pure of which also had the least affinity for tumours (Schwartz et al 1955). He made new preparations from the other fractions in order to isolate the more active components and eventually produced an acetic-sulphuric acid porphyrin which has come to be known as haematoporphyrin derivative (HpD).

With this new agent, Lipson and colleagues at the Mayo Clinic localised tumours in the bronchus, oesophagus and cervix, achieving good correlation between fluorescing sites and biopsy-proven cancers (Lipson et al 1960, 1961, 1964).

It wasn't until the 1960s that photodynamic therapy with HpD was used to treat tumours in humans. The first use of HpD to treat a large recurrent breast tumour was reported in a meeting in 1966 (Lipson et al 1996) and a single case of bladder carcinoma was reported in 1975 (Kelly et al). The first systematic trials in humans were initiated by Dougherty and colleagues in 1978 on a group of 25 patients, achieving complete or partial response in 111 of 113 cutaneous or subcutaneous malignant lesions (Dougherty et al 1978). The tumours included metastases from carcinomas of the breast, colon, prostate, squamous cell, basal cell and endometrium, malignant melanoma, mycosis fungoides, chondrosarcoma and angiosarcomas. It is interesting to note Dougherty includes malignant melanoma. One can only assume that this must be an amelanotic type, as light does not penetrate black melanotic tissue.

An important finding was that no tumour type was unresponsive to treatment: when applied correctly, photodynamic therapy will always produce necrosis.

Since these important early studies, HpD was investigated and found to be effective in a wide variety of tumours, using various light doses and sources (Misazumi et al 1983, Hayata et al 1982, Thomas et al 1987, Ward et al 1982). Although there was much room for improvement in the development of better photosensitisers and light sources, the principle of photodynamic therapy had been firmly established.

The next chapter describes in more detail the mechanisms of the photodynamic process and reviews the development of more pure and more active photosensitisers.

Chapter 4: Current Status of Photodynamic Therapy

4.1 Basics of Photodynamic Therapy

The three fundamental elements of PDT have been identified as oxygen, a photosensitiser and light. The photosensitiser is excited and activated by the light and interacts with molecular oxygen to produce an excited state - singlet oxygen - which is highly cytotoxic with a short lifetime ($<0.04\mu\text{s}$) and a short radius of action ($<0.02\mu\text{m}$). This reaction - called a type II reaction - is the most frequent photochemical pathway to cell destruction (Athar et al 1989, Grossweiner et al 1982), but the activated sensitiser may also react directly with parts of the cell structure causing the release of free radicals which then react further with molecular oxygen (type I). The initial photochemical reaction produces further radical chain oxidative reactions and a cascade of cell damage that make it difficult to isolate the initial site of action.

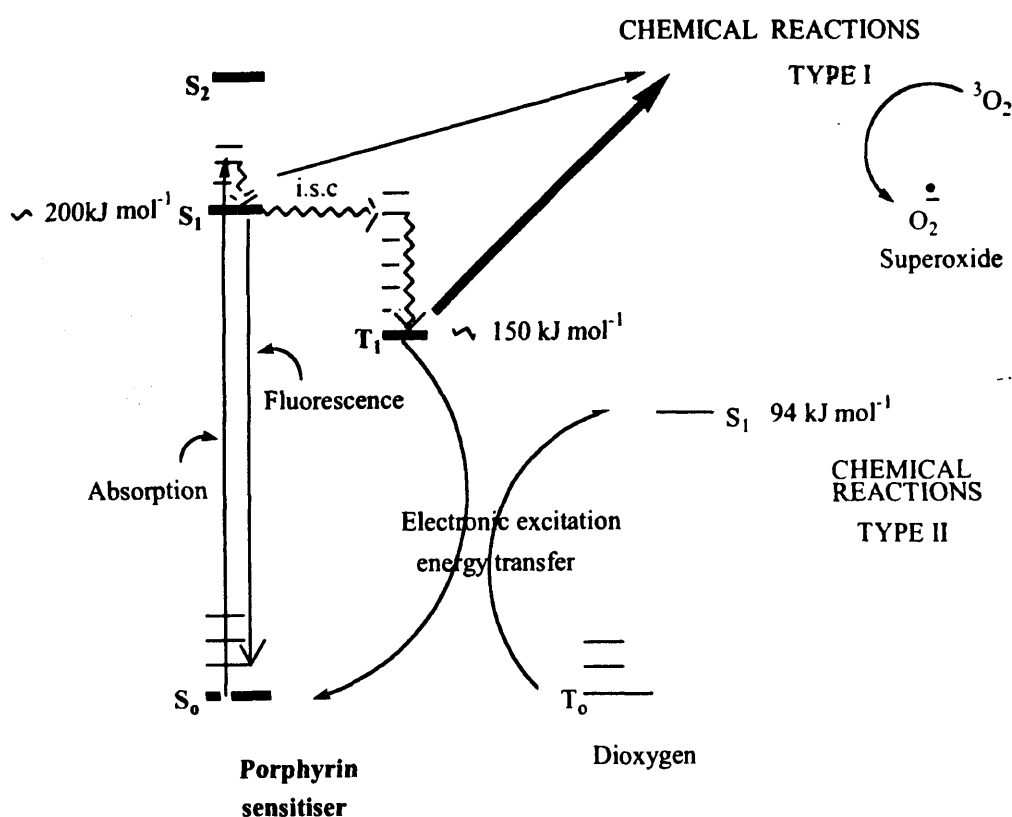


Fig 4.1: The principles of photodynamic therapy: Type I and Type II reactions
[source: Bonnett 1995 p20, fig 2]

The direct cytotoxic activity and microvascular damage contribute to the destruction of tumour cells, which is manifested as swelling and formation of necrotic tissue (Foote 1990). This eventually sloughs away (or is re-sorbed) and is followed by normal healing and re-epithelialisation of the treated site.

The importance of oxygen in the phototoxic process is illustrated by the observation that hypoxic cells respond less well to photodynamic therapy (Pass 1993, Denekamp 1992, Fingar et al 1992). Oxygen requires 94 kJ/mol to raise it from the triplet ground state to the excited singlet state. In photodynamic therapy this energy is acquired from the photosensitiser which has itself been raised to a high energy state. Only light-absorbing compounds which can emit energy greater than 94kJ/mol are therefore capable of activating ground state oxygen. This corresponds to an absorption wavelength of around 850nm: photosensitisers are therefore only suitable for photodynamic therapy if their activation wavelength is below this figure.

4.1.1 Mechanisms of Cell Killing

The mechanisms by which tumour cells are killed have been extensively researched (eg Lofgren et al 1994 and 1995, Foote 1990). Photosensitisers tend to accumulate in the mitochondria, lysosomes, and plasma membranes. Photosensitisers that localise in the cellular membranes have a direct cytotoxic effect which is noticeable within minutes of light exposure. On a light microscopy scale this can be seen as swelling and bleb formation. At a sub-cellular level there is shedding of vesicles containing plasma membrane marker enzymes, depolarisation of the membrane and general collapse of the ionic balance and cell structure.

Photosensitisers such as Photofrin® and 5-ALA which accumulate in the mitochondria may also achieve their photochemical effect through apoptosis. Apoptosis is a mechanism whereby organisms initiate a programmed cell death - or 'suicide' - resulting in fragmentation of nuclear DNA and dissociation of the cell into membrane-bound particles that are absorbed by other cells. Malignant cells often cannot undergo apoptosis which perhaps explains their resistance to chemotherapy (Vaux et al 1996, Samali et al 1996).

Photodynamic therapy can induce an apoptotic response (Agarwal et al 1991) through the release of cytochrome c and other mitochondrial factors, which bypasses the normal programmed cell death mechanisms. Apoptosis can therefore be induced even in those malignant cells that have lost the ability to initiate their own cell death.

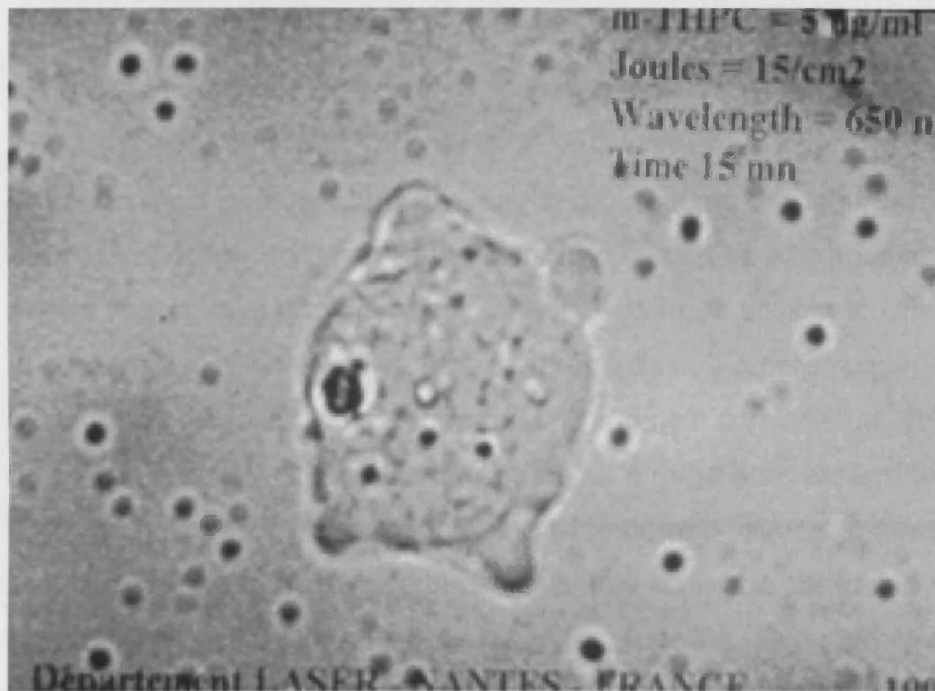


Fig 4.2: PDT induced apoptosis showing blebbing of the plasma membrane with vesicle formation and karyorrhexis (discontinuity of the nuclear envelope) (T Patrice, Nantes, France 1998)

The vascular effects of photodynamic therapy are also important in tumour control. The mechanisms differ according to which photosensitiser is used and include platelet activation and release of thromboxane, increased vascular leakage, platelet aggregation and blood flow stasis. The end result is the same: the microvasculature is damaged and tumour hypoxia results (Star et al 1986).

It is now generally believed that if light activation is carried out at a short interval following drug administration (the drug-light interval) cell death results from microvasculature shutdown with subsequent tumour anoxia and necrosis (Korbelik et al 1994, van Geel et al 1996, Wang et al 1997). If the drug-light interval is longer, cell death is achieved either via membrane rupture or apoptosis (Luo et al 1996, Kessel et al 1997, Ketabchi et al 1998).

Research suggests that there may also be an immune response generated by photodynamic therapy (Korbelik 1996 and et al 1997). Korbelik et al claim that photodynamic therapy induces inflammation at the treated site, which causes invasion of activated myeloid cells. The implications of this are highly significant as it may be that application of photodynamic therapy to a local tumour could induce cell death in distant metastatic cells.

4.1.2 Selectivity of Effect

Although most photodynamic therapy treatments are carried out by the application of light to a very well-defined area such as a tumour or other lesion, one of the goals of photodynamic therapy was - and still is - to develop photosensitisers which will collect preferentially in the cells requiring treatment, while being completely cleared from surrounding normal tissue. It would then be possible to shine the activating light over a large area around the tumour, inducing necrosis in the tumour, but leaving normal tissue unharmed. In addition to simplifying routine treatments, this would be particularly useful in the treatment of multiple or poorly differentiated lesions. These present a particular challenge to the surgeon due to the difficulty in identifying the tumour margins and in removing rapidly advancing 'threads' of tumour cells that may extend far beyond the main tumour bulk.

There is evidence that photosensitisers are taken up and retained preferentially by neoplastic tissue but as yet this effect is not sufficiently pronounced to allow for a completely selective clinical response. If sufficient light is delivered then any tissue will be destroyed. The concentration difference between tumour and normal tissue ranges from 4:1 to over 12:1 for different photosensitisers (Ronn 1999; Lofgren et al 1994). The highest tumour to normal tissue ratios are generally found in brain tissue (Kostron et al 1996).

The mechanisms of preferential distribution of photosensitisers in tumours are not yet fully understood and a number of explanations for the effect have been suggested. Probably more than one mechanism is involved. Simple pooling and retention of the photosensitiser could take place due to the large interstitial spaces and poor lymphatic network in tumour tissue (Henderson et al 1992).

Another suggestion is that the increased density of lipoprotein receptors in tumour cells could increase uptake of LDL-bound photosensitiser (Kessel D 1986). This is clearly not the only mechanism as some photosensitisers that associate well with lipoproteins are poor tumour localisers; while other photosensitisers that are excellent tumour localisers have a poor affinity for lipoproteins.

A major focus of current research is to develop photosensitisers with increased selectivity for tumour cells.

As activated photosensitisers may also fluoresce while returning to the ground state, selective accumulation can be exploited in the fluorescence detection of tumours allowing a one-step process of detection and treatment.

4.1.3 Depth of Effect

Most photosensitisers are administered systemically, although some can be applied topically in the treatment of skin cancer and dermatological conditions. After a period of time to allow maximal accumulation of the photosensitiser in the target tissue, low power light of the appropriate wavelength is directed onto the tumour. The depth of effect which can be achieved depends on the absorption peaks of the photosensitiser employed. Photofrin® (porfimer sodium) for example has a number of absorption peaks between 400nm and 650nm; benzoporphyrin derivative has a maximum absorption peak at 690nm and Foscan® (temoporfin, mTHPC) has a strong absorption peak at 652nm.

As light passes through human tissue, a proportion is lost through scattering, reflection and absorption. This rate of loss per unit of thickness is called the coefficient of extinction and varies with the type of tissue and the wavelength of the light. Human tissue transmits light most effectively in the red part of the visible spectrum (Figure 4.3), and current research is directed towards developing photosensitisers with longer activation wavelengths which can be activated in this region, allowing for a greater depth of photodynamic effect. Photofrin® is usually activated at 630nm achieving a maximum depth of effect of 0.5cm (Gomer et al 1984, Wilson et al 1985) while temoporfin activated at

652nm has a depth of effect ranging from 1 to 1.5cm (Lofgren et al 1994, Berenbaum 1986b, Ris et al 1991).

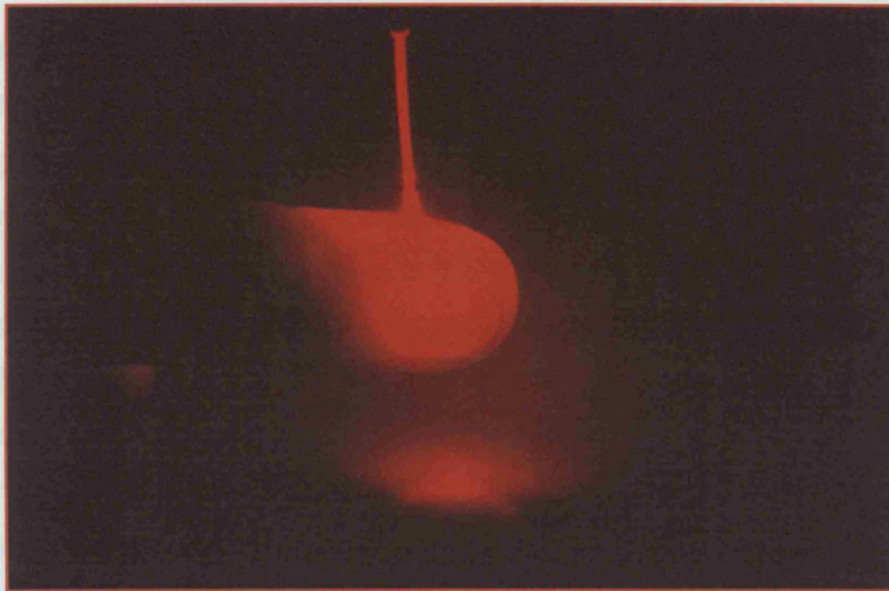


Fig 4.3: Transmission of light by human tissue

As most photosensitisers have more than one absorption band, this property can be exploited to control the depth of effect for various clinical applications by using different activating wavelengths. For example, temoporfin activated by green light at 514nm produces a depth of effect of 0.5cm.

4.1.4 Effect on Underlying Structures

As PDT is a cold photochemical process, there is no tissue heating involved, so it would be expected that connective tissues such as collagen and elastin would be largely unaffected. Barr et al (1987a) have demonstrated that collagen is preserved in the sub-mucosal layers of the rat colon treated with PDT using a sulphonated aluminium phthalocyanine. Although cross-linking of collagen fibres can be induced by singlet oxygen photo-oxidation, this process does not appear to compromise the mechanical properties and may even increase its resistance to deformation (Verweji et al 1981).

Endothelial cells have been shown to be highly sensitive to PDT injury, and indeed it has been suggested that this is directly responsible for the tumour necrosis observed following PDT (Berenbaum et al 1986a and 1990, Zhou et al

1988, He et al 1991). Chaudhuri et al (1987) found that endothelial cells were more susceptible to PDT injury than tumour cells.

Photochemical injury results in a different type of injury from that observed with heat injury created by thermal lasers or diathermy. This non-thermal photodynamic injury does not damage or denature the sub-epithelial collagen and elastin, and the preservation of non-cellular supporting elements has been demonstrated, which suggests that the tissue architecture may be maintained while cellular and vascular elements are damaged. This may provide a matrix for regeneration of normal tissues and account for the excellent healing observed in most animal studies. The indications are that there is much less risk to the integrity of underlying structures than with thermal laser techniques and surgery.

Part of the research programme described in this thesis was directed at an assessment of the effect of photodynamic therapy on arteries (see Chapter 6).

4.1.5 Light Dose

The aim of photodynamic therapy is to disperse low power light over the surface area (or into the volume) of the tumour to initiate the photochemical process without inducing side effects such as thermal damage. This is in contrast to surgical laser treatments in which the light is used to cut or coagulate or for photoacoustic effects.

The light dose (or fluence) is expressed in terms of joules per unit of area: J/cm^2 and the rate at which this light is delivered (the fluence rate) is expressed in milliwatts per unit area: mW/cm^2 . This is relevant clinically as there is a limit to the rate of energy delivery which can be delivered to normal tissue before unwanted thermal effects are induced. Photofrin® typically requires light energies of 25 to $300\text{J}/\text{cm}^2$ to induce photoactivation for surface treatment and for interstitial treatment 100 to 400J for point sources or 100 to $400\text{J}/\text{cm}$ for diffuser applications. Foscan® on the other hand can be activated with light doses of less than $20\text{J}/\text{cm}^2$.

The fluence rate is an important factor in the efficiency of tumour response to PDT. At high fluence rates, it is hypothesised that oxygen consumption can outpace the rate of oxygen diffusion from surrounding capillaries resulting in a decrease of oxygen in the treated area and a reduced response (Foster et al 1991). High fluence rates (over 150 mW/cm²) also cause tissue heating so that a combination of PDT plus thermal effects results. Lowering the fluence rate has been shown to improve tumour response but also increases treatment times.

Reports from several centres have suggested that PDT effects might be enhanced by fractionating the light (van der Veen et al 1995, Foster et al 1991, Pe et al 1994, Messmann et al 1995). Using ALA, Van der Veen et al (1995) found that a 75-minute period between light treatments enhanced the effect (although the total light dose was higher in the fractionated dose experiments). Messmann et al (1995) found that a single break of 150 seconds during a 250-second exposure was capable of increasing the area of a PDT-induced ulcer in normal rat colon by a factor of 5 compared with the same total light dose administered without a break. They suggested that the break permitted re-oxygenation of the tissue, therefore when the light was applied for the second fraction, there was more oxygen available for a PDT effect.

The time at which the break was made markedly influenced the effect (the effect was greater with an earlier break) but there was no advantage in having more than one break. It is not clear from this work whether fractionating the light increased the maximum area of necrosis achievable or just made it possible to achieve the same area of necrosis with a smaller total light dose.

4.1.6 Treatment Times

Treatment times vary considerably and are related to the absorption of light by the photosensitiser and the efficiency of conversion of the light to oxygen.

As was described in the introduction to this section, only those sensitisers that can emit more energy than 94kJ/mol are capable of activating singlet oxygen. The most efficient photosensitiser therefore would be one which yields one

molecule of singlet oxygen for each photon absorbed - the quantum yield. The more efficient photosensitisers have a higher quantum yield: Photofrin® has a quantum yield of around 0.5 and is not therefore a particularly active photosensitiser whereas Foscan® is a more efficient generator of singlet oxygen with a quantum yield of around 0.87.

In order to achieve the desired level of tissue necrosis, Photofrin® requires treatment times which can be unacceptably long if large areas have to be treated. Typical treatment times for these earlier sensitisers are 15 to 40 minutes; newer photosensitisers such as Foscan® can induce effective cell killing with the application of around 200s of low power laser light.

4.1.7 Side Effects of Photodynamic Therapy

All photosensitisers currently in use or in development are inherent of very low systemic toxicity in the absence of light. However, systemic administration of the photosensitiser leads to a period of unwanted residual photosensitivity that must be managed until the drug is eliminated. Exposure to sunlight before the photosensitiser has been eliminated causes a reaction which resembles sunburn but is in fact simply a continuation of the photodynamic reaction. If exposure continues for long enough, blistering and full thickness necrosis will occur. During faulty administration, high concentrations of photosensitiser can build up in extravasated areas. These must be covered with opaque dressings to avoid serious burns. The earlier photosensitisers have a residual photosensitivity of eight to ten weeks; this has been reduced to two weeks with the newer agents and is expected to decrease still further as new photosensitisers are developed.

While residual photosensitivity is a problem and can be inconvenient to the patient, it is of relatively little significance compared to the adverse effects of chemotherapy and radiotherapy.

Unlike treatment with laser ablation, the light used in PDT is 'cold'. However, a significant number of patients complain of a burning sensation during treatment with power densities of up to 100mW/cm² despite the fact that measurements of

skin temperature during treatment have recorded no increase (McCaughan 1999). The burning sensation is probably due to the photodynamic process rather than any thermal effects.

Since most photodynamic therapy photosensitisers do not accumulate in cell nuclei, photodynamic therapy is unlikely to cause DNA damage, mutations or carcinogenesis (Moan 1986). This is in direct contrast to cytotoxic chemotherapy, which is specifically designed to interfere with cell replication.

4.1.8 Light Sources

Light emitting diodes (LEDs) and xenon lamp sources are commonly used for dermatological applications, but lasers provide the most convenient and controllable light source. Laser light is coherent and monochromatic and can be directed along fibre optic cables, allowing light to be introduced into hollow organs and deep-seated tumours.

A number of metal vapour and tuned-dye lasers are available which can be adjusted to emit over a range of wavelengths appropriate to various photosensitisers. These are expensive and large but do have the advantage of being tunable to various wavelengths, which may become a more valuable characteristic when photodynamic therapy is a mainstream treatment in PDT units employing a number of photosensitisers.

Diode lasers, which are much less expensive and more portable than metal vapour or tuned-dye lasers, have become an attractive light source. Multiple low power diodes are used in phased arrays to produce a high power light beam. Diode lasers operating in the region of 660 to 700nm and others operating in the region of 780 to 850nm are now readily available.

The laser beam is usually guided through a fibre attached to a microlens to allow for uniform irradiation. Spherical or cylindrical diffusing tips are usually used for direct insertion of the fibre into a tumour mass.

4.2 Photosensitisers

Most photosensitisers - with the exception of 5-aminolaevulinic acid are composed of interlinked pentacyclic hydrocarbon rings which resemble the basic porphyrin structure (the tetrapyrrole group). While the porphyrin skeleton is not a prerequisite for photodynamic activity, the advantages of this structure are that it seems to lead to compounds that are efficient generators of singlet oxygen when activated by light; are usually non-toxic in the absence of light; have good to strong absorption bands in the red part of the visible spectrum and are reasonably stable.

The main compounds currently in development, are the macrocyclic tetrapyrroles – porphyrins, chlorines (similar to chlorophyll α), purpurins, benzoporphyrins, and naphthalocyanines.

The four photosensitisers used in this research programme are described below.

4.2.1 Photofrin® (porfimer sodium)

More than one hundred years after the isolation of the first haematoporphyrin, Photofrin®, a pure and reasonably consistent derivative, was made available commercially by QLT, Canada. Photofrin® was developed by Dougherty et al (1984) and is the most active photosensitiser component in haematoporphyrin derivative. Even in this purified state, the commercial preparation contains around 20% of inactive monomers and 80% of the active porphyrin dimers and oligomers.

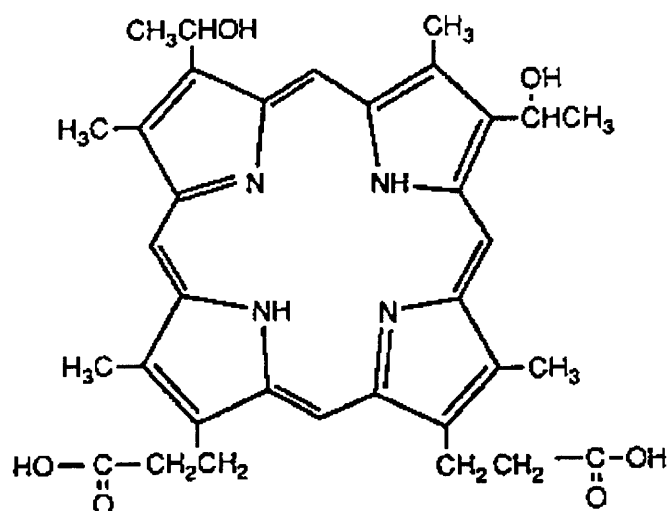


Fig 4.4: Structure of Haematoporphyrin [source after Bonnett 2000]

It is given intravenously at a dose of 2 to 5mg/kg bodyweight and circulates in a number of forms: as unbound aggregated components; bound to albumin and globulins (monomer); and lipoproteins (dimers and oligomers) (Jori 1989). The active component of Photofrin® is mostly deposited in the mitochondria, lysosomes and plasma membrane.

The optimum drug-light interval is one to two days and although the drug has a strong absorption peak in the blue/violet region at 405nm, the weakest peak at 630nm is usually used in order to maximise the penetration of light into human tissue (Gomer et al 1984, Wilson et al 1985).

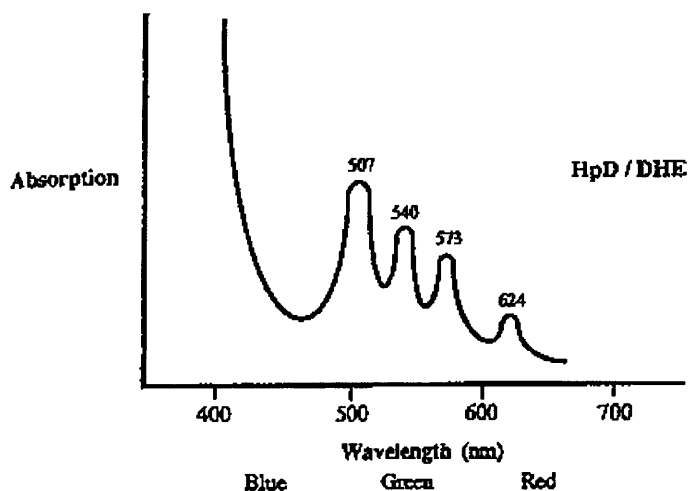


Fig 4.5: Absorption peaks [Axcen Pharmaceuticals Inc].

At this wavelength (650nm), the penetration of light is in the range of 1 to 5mm (Driver et al 1991, Wilson 1986) therefore the achievable depth of effect with Photofrin® is limited to 0.5cm.

Relative uptake between tumour and normal tissue is not high being in the region of 4:1 with no discernible difference in for instance the pancreas. Residual cutaneous photosensitivity is around eight to ten weeks (Dougherty et al 1978, Oseroff 1993, Pass 1993). The efficiency of conversion of light to cytotoxic products (the quantum yield) is moderate (~0.5) and treatment times are therefore relatively long.

Despite these limitations, Photofrin® is a useful drug that established the credentials of photodynamic therapy as a valid treatment for a wide range of malignant and non-malignant conditions. It is licensed in various countries for the treatment of early and late-stage lung and oesophageal cancer and for gastric and cervical cancer, and is being studied in other areas such as Barrett's oesophagus, cancer of the larynx and prostate, skin cancers and metastatic cutaneous and subcutaneous breast cancers.

4.2.2 5-Aminolaevulinic Acid

Instead of administering an exogenous porphyrin, 5-aminolaevulinic acid has been used to generate endogenous protoporphyrin. 5-aminolaevulinic acid

(ALA) is a naturally-occurring precursor of haem whose production is controlled by negative feedback inhibition of the end product on the enzyme ALA synthase (Rimington 1966). However, if excess exogenous ALA is given, this feedback control is bypassed, leading to a build-up of protoporphyrin IX (PPIX) (Divaris et al 1990). Since PPIX is an effective photosensitiser (Malik et al 1987) this mechanism makes it possible to sensitise cells capable of haem synthesis. Because administration of ALA only temporarily overloads the natural synthetic pathway, photosensitisation lasts no more than several hours.

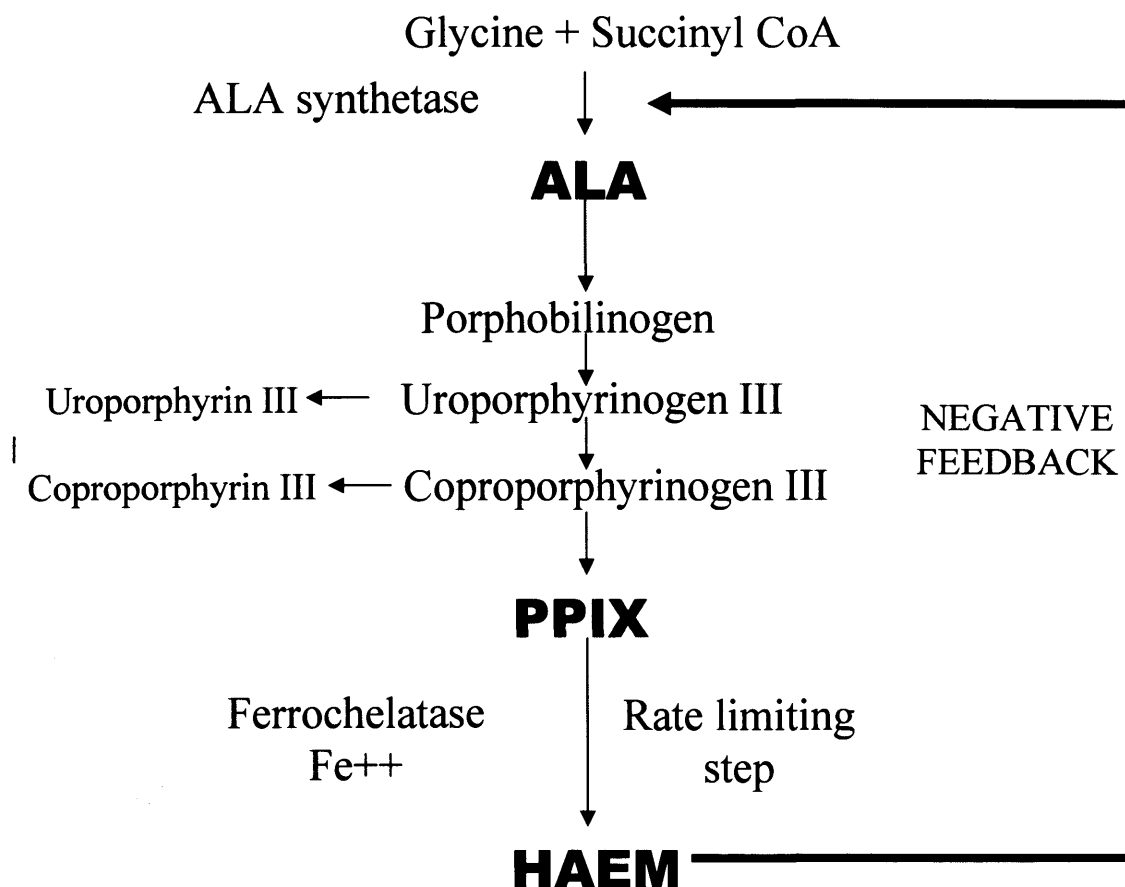


Fig 4.6: The haem synthetic pathway – administration of ALA results in an increase of PPIX

ALA has an optimal drug-light interval of three to six hours and requires a light dose of around 50J/cm² delivered at 630nm, but it can also be activated by green light at 543 to 548nm (Fritsch et al 1997) and blue light.

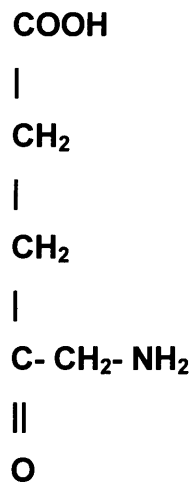


Fig 4.7: Structure of 5-aminolaevulinic Acid

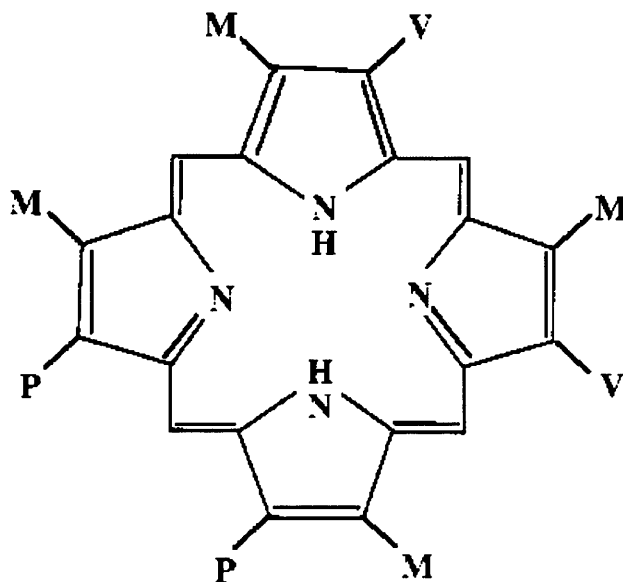


Fig 4.8: Structure of Protoporphyrin IX. [source: Bonnett 1995 p26]

A major advantage of ALA is that, even after systemic administration, cutaneous photosensitivity lasts at the most one to two days (Mustajoki et al 1992). Tissues treated with ALA photodynamic therapy heal remarkably well, making it feasible to treat extensive superficial lesions in the mouth (Grant et al 1993b).

PPIX has been shown to accumulate more in the epithelium than in the underlying muscle of hollow organs like the GI tract (Loh et al 1993b) with the consequent possibility of selective damage to this layer following PDT.

ALA can be delivered in topical, oral or intravenous formulations. The maximum dose of ALA tolerated by mouth may be only just above the threshold dose for producing a PDT effect (ie 60mg/kg). Intravenous formulations halve the dose required to achieve specific tissue levels (30mg/kg), therefore the first pass metabolism in the liver which leads to increased transaminase levels associated with oral administration (Regula et al 1995) is avoided and the maximum tolerable dose may be higher.

The uses of 5-aminolaevulinic acid are constrained by its depth of effect (<0.2cm) to superficial lesions. 5-aminolaevulinic acid has been licensed for the treatment of actinic keratosis and has shown good results in the treatment of basal cell carcinoma (Kennedy et al 1990, Peng et al 1995, Calzavara-Pinton 1995, Wennberg et al 1996). Areas under investigation include the oesophagus (Gossner et al 1998), metastatic breast cancer to the skin (Cairnduff et al 1994) and superficial urothelial cancer (Peng et al 1997).

4.2.3 Sulphonated Aluminium Phthalocyanine

The phthalocyanine structure is similar to the porphyrin configuration but with some differences which shift absorbance to the 650 to 700nm region. Phthalocyanine and the related naphthalocyanines have intense absorbance in the red region, with the naphthalocyanine absorbance band reaching as high as 800nm. Due to the high extinction co-efficients, some phthalocyanines are very potent PDT agents. They are conjugated with a number of metals, the most efficient being zinc and aluminium which extend the triplet state lifetime.

Disulphonated aluminium phthalocyanine given in typical doses of 0.5 to 1.5mg/kg is illuminated at 675nm and light is delivered between 150 and 600J/cm² at a fluence rate of more than 50mW/cm². This is a high light dose, which leads to relatively long treatment times for any but the smallest tumours.

Disulphonated phthalocyanine has been shown to be a highly potent sensitiser *in vivo* in a variety of animal tissues (Chatlani et al 1991, Nuutinen et al 1991, Loh et al 1992). They have been extensively investigated by Stranadko et al (1994 and 1997) in various clinical applications such as gynaecological and oral

malignancies. However, despite extensive use in Russia, this group of drugs has not been used in human studies in the UK. Animal studies suggest a similar effect to Photofrin® but with a marked reduction in photosensitivity post administration.

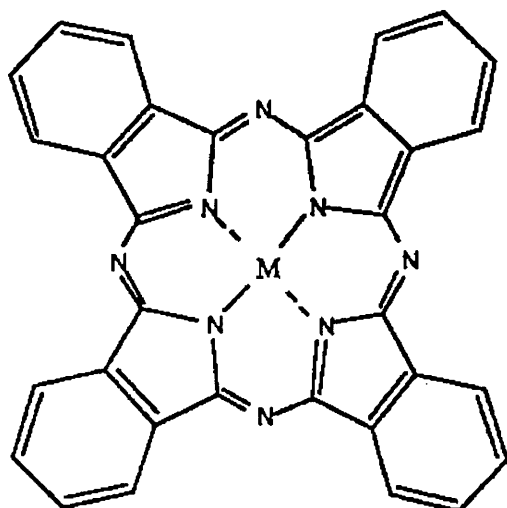


Fig 4.9: Structure of Pthalocyanine [source: Bonnett 1995 p29]

4.2.4 Tetra(m-hydroxyphenyl)chlorin, mTHPC, Foscan®

Foscan® belongs to a group called the chlorins in which the double bonds on the porphyrin rings are progressively saturated. Chlorin has one double bond of porphyrin reduced, bacteriochlorin has two bonds reduced. This results in a shift of the absorption peaks towards the visible red region. Foscan® is a single compound of 98% purity (Wagnieres et al 1998) and is isometrically pure. The drug is only sparingly soluble in water and therefore requires formulation to obtain an injectable product.

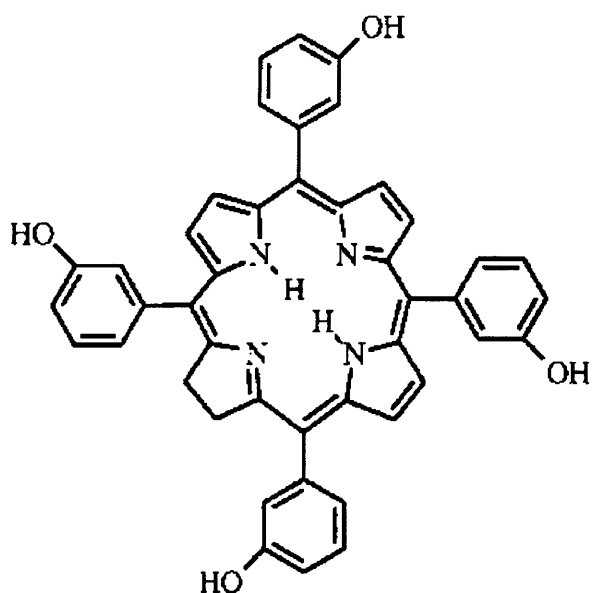


Fig 4.10: Structure of mTHPC [source: Bonnett et al 1989]

Major absorption peaks for Foscan® are at 652 and 514nm. Red light of 652nm penetrates human tissue better than at 630nm, so a greater depth of effect can be obtained. It is a little unclear why such an enhanced depth of necrosis is obtained with such a small wavelength shift so generating tissue necrosis of over 1.5cm.

The optimum drug-light interval is four days and the usual dose for therapeutic PDT is 0.15mg/kg. It preferentially accumulates in tumour tissue to a concentration of up to twenty times normal tissue. Early clinical work with PDT on the tumour bed after resection of mesotheliomas showed that a dose of 0.3mg/kg mTHPC, activated at 650nm with a dose of 10 J/cm² could produce 10 mm depth of necrosis with good healing.

Residual photosensitivity is in the region of two weeks (Wagnieres et al 1998).

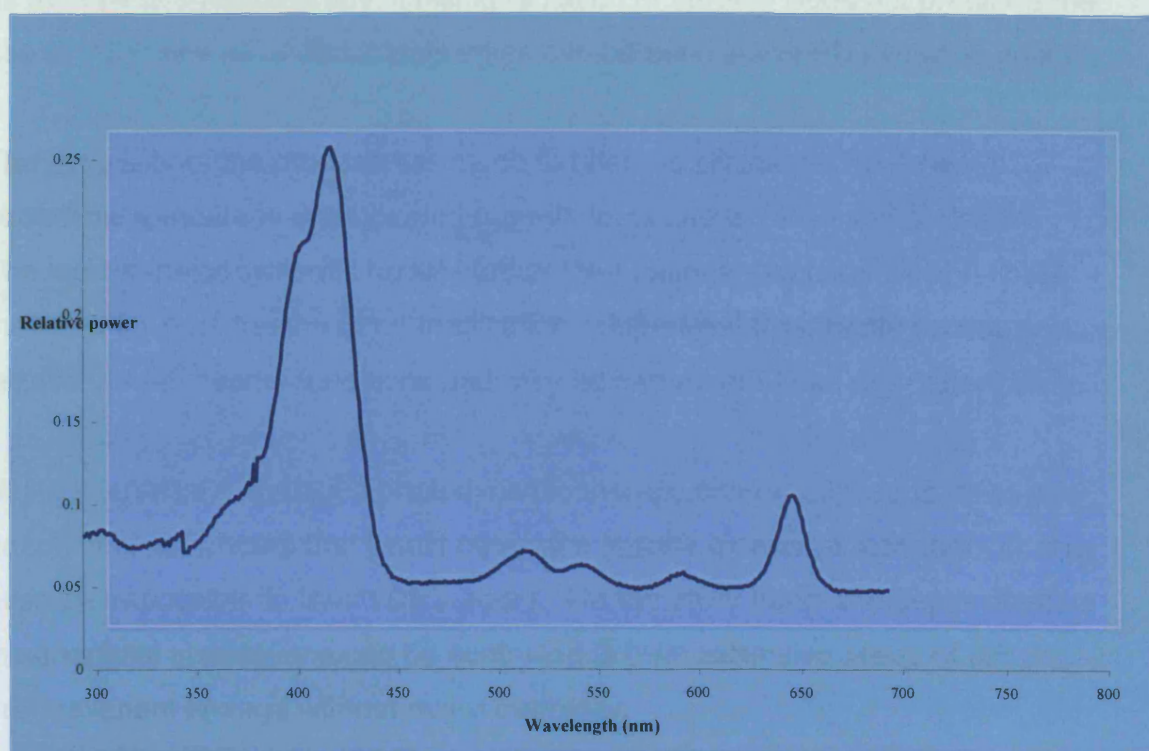


Fig 4.11: Absorption peaks for mTHPC

Foscan® is the most active of all photosensitisers currently undergoing investigation, with a quantum yield of singlet oxygen of ~ 0.87 when activated at 652 nm. It has a low activation energy at 652 nm requiring light doses as low as 10 J/cm^2 for photodynamic effect, although the usual light dose is 20 J/cm^2 with a fluence rate of 100 mW/cm^2 .

Drug doses are also correspondingly low, being only 0.1 mg/kg to produce a therapeutic effect. Treatment times under these conditions are typically in the order of 200s. A study on the murine-RIF-1 tumour showed that Photofrin® requires a light dose 4 to 13 times higher than that required by Foscan® for a similar anti-tumour effect (van Geel et al 1995).

4.3 Advantages of Photodynamic Therapy

Photodynamic therapy (PDT) is a relatively new approach to the treatment of malignant conditions. It is minimally invasive and unlike ionising irradiation, can

be carried out repeatedly at the same site. It has particular appeal in oncology as the use of chemotherapy, ionising radiation or surgery does not preclude the use of PDT, and all of these techniques can be used in the PDT-treated patient.

The simplicity of the process has much to offer the clinician. Treatment of superficial tumours is often carried out with local anaesthesia and sedation. The lack of major systemic toxicity (other than residual photosensitivity) limits the need for post-treatment co-medication. Superficial treatments do not require sterile theatre conditions and may be carried out in an out-patient clinic.

Using interstitial techniques photodynamic therapy can be applied to large and deeply buried tumours that would otherwise require extensive resection, or may even be impossible to treat with surgery. On the other hand, the depth of effect in superficial applications can be controlled to treat extensive areas of diffuse pre-malignant change without major morbidity.

It appears that every type of tumour cell, whether mesodermal, endodermal or ectodermal in origin is sensitive to the PDT process. The problem of multi-drug resistance, which is a serious limitation in conventional oncology, does not apply to PDT; even cell lines showing multi-drug resistance can be killed by PDT (Stewart 1999).

4.4 Potential Applications of PDT in the Oral Cavity

Pre-malignant dysplastic lesions or non-invasive cancers are common in the mucosa of the aerodigestive tract. Treatment of a pre-malignant lesion before it becomes invasive is clearly desirable and preferable to treatment of a solid malignancy. Eradication of the lesion at this stage will also minimise the risk of metastatic disease. However, current treatments for pre-malignant conditions are far from satisfactory (see Chapter 2). Surgery requires general anaesthesia and carries significant morbidity including functional impairment and disfigurement. Radiation therapy is effective but is rarely justified in terms of the acute and long-term adverse effects and can typically only be administered once.

Photodynamic therapy is emerging as an attractive treatment for mucosal dysplasia and CIS as a wide area of mucosa, including areas of apparently normal mucosa, can be treated superficially.

The studies described in the remainder of this thesis were designed to investigate the safety of PDT and its efficacy in early and advanced disease of the oral cavity.

Despite all of the potential mechanisms of cell death following PDT there is no doubt that the chemical reaction in response to singlet oxygen production is a very rapid event following illumination. Some of the mechanisms may be by apoptosis and necrosis, which are dependent on variable drug dose, light dose interval and light dose as well as the physiology of the tumour. Some tumour effects seem to be related to microvascular damage with some subsequent anoxia. However, as cell death is of rapid onset following PDT it is not really necessary to speculate on events that occur beyond the immediate illumination time to explain the outcome of treatment (Thibaut et al 2002).

Chapter 5: Description of the Research Programme

5.1 Introduction

The overall aim of the research programme at UCL was to define the clinical response of normal and malignant tissues in the oral cavity to photodynamic therapy using a number of different photosensitisers, and in conditions ranging from pre-malignancy through early disease to advanced disease. Two initial safety studies were carried out, followed by clinical studies in which the intention was both curative and palliative. The four photosensitisers used in the programme were 5-aminolaevulinic acid, Photofrin®, di-sulphonated aluminium phthalocyanine and Foscan®, although to date I have not carried out any human treatments with di-sulphonated aluminium phthalocyanine.

At the start of this clinical programme, there had been a number of studies carried out on animal models, but very little clinical work. The main body of experimental work relevant to oral cavity cancer was carried out by Maurice Meyer (Meyer et al 1991), who studied the effects of disulphonated aluminium phthalocyanine PDT on the oral mucosa and other tissues of the rabbit. He designed an ingenious series of experiments using the *socket of an extracted lower incisor tooth of the rabbit*. By passing a laser fibre along the socket (which is about 4cm long), he was able to carry out a series of treatments in which the mandible, salivary gland tissue, muscle, cartilage and oral mucosa were illuminated.

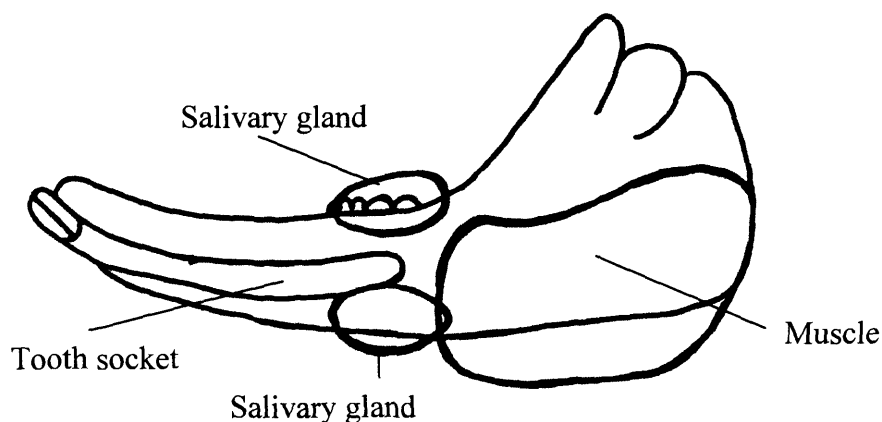


Fig 5.1: Experimental set-up for PDT effects on normal rabbit mandible and surrounding tissues (Meyer et al 1991)

A number of rabbits received disulphonated aluminium phthalocyanine PDT at varying doses and with varying light doses. Some control rabbits received drug and no light and others received light and no drug. After serial sacrifice Meyer made a number of important observations of the histological effects of disulphonated aluminium phthalocyanine PDT on normal oral tissues, which are summarised below.

Tissue treated	PDT effect
Mucosa / gingival	Sensitive at low doses. Necrosis with complete regeneration.
Muscle	Dose-related, sensitive at high doses. Necrosis with scarring
Salivary gland	Dose-related, sensitive at high doses. Some necrosis with scarring
Nerves	Little effect
Bone	Little damage. Slight delay in healing
Cartilage	No effect

Table 5.1: Summary of PDT effects on normal rabbit mandible and surrounding tissues (Meyer et al 1991)

Although this was a study on normal tissue, there was little selectivity of effect between different tissues. However, the key finding was that almost all tissues healed or regenerated well following PDT. In particular, mucosa underwent consistent full thickness necrosis, but healed without any noticeable scarring. This of course has major implications for the treatment of large areas of oral mucosa, as it suggests that it would be possible to achieve healing without any disruption of the mucosal surface. The damage to the salivary glands was of some concern, but this was to some extent reversible and merely localised to the area of illumination and bone cartilage and nerves were relatively unaffected.

5.2 Other Safety Studies

A small number of other safety studies had been carried out. Suzuki et al (1987) described endothelial cell loss in aortas of rats treated with HpD PDT. They observed no other damage to the vessel wall and found that endothelial cell regeneration occurred within five days. The thickness of these large vessels may have allowed preservation of the endothelium along the posterior surface of the aorta due to limited light penetration, allowing the opportunity for endothelial regeneration in a short time.

Studies of PDT-treated rat colons (Barr et al 1987b) and rat tracheas (Smith et al 1993) have shown preservation of mural collagen and no reduction in the hydrostatic pressure required to rupture the treated organ. These studies clearly demonstrated that the PDT effect was generally well tolerated by normal (animal) tissue, but the extent of experience at this stage was very limited. PDT effects on skin had been studied in the 80s by, for example Benstead and Moore (1988) showed skin necrosis was dependent on the size of the illumination spot and that shielded tissues provided capillaries to grow into treated areas and prevent necrosis

5.3 Clinical Studies

At the start of this programme (in 1991), there was little published clinical data for head and neck cancer. While the work carried out by Hayata and Kato (1982, 1984 and 1993-Hayata only) in Japan using haematoporphyrin and Photofrin® had demonstrated a clear use for PDT in the management of lung cancer, the situation with head and neck cancer was less clear. Zhao in Beijing reported a series of 72 patients with oral squamous cell carcinoma using haematoporphyrin derivative (Zhao et al 1991). While the results were very impressive, there was a huge variation in drug and light dose used with no clear indication how or why the treatment parameters were chosen.

Wenig and Gluckman had just published their experiences with HPD and Photofrin®. These studies were more consistent in their design with light doses of 50 or 100J/cm² (Gluckman 1991b) and 125J/cm² (Wenig et al 1990). The

numbers at specific anatomical sites and the stages of disease were varied so that numbers of patients in each group were small. However, the results of treating T1 and T2 oral cavity cancers were certainly encouraging with initial complete response rates of 77% (Wenig 1990) and 85% (Gluckman 1991b).

5.4 Research Programme

The above is a brief outline of the state of PDT knowledge at the time of setting up the programme. Clearly there were a number of unanswered questions about normal tissue tolerance to PDT and there was almost no scientific rationale for treating head and neck cancer in humans. Sporadic clinical reports had appeared in the literature, but there was no standardization of treatment parameters. The goal of the research programme carried out at UCL was to establish the safety and efficacy of PDT as a treatment for oral cancers. The studies described below were carried out.

5.4.1 Animal studies to Determine The Effects of Photodynamic Therapy on Vital Structures

Several studies were undertaken under my co supervision to determine the ability of normal tissue to tolerate PDT treatment. The most important of these were on major blood vessels in the neck

5.4.2 Pilot Studies to Assess Photodynamic Therapy Effects on Human Tissue

In this series of studies, we tried to evaluate the PDT effects on (human) oral cancer and the surrounding normal tissue. This was done with a series of treat and excise and treat and biopsy studies. Three sensitisers were used Photofrin®, Aminolaevulinic Acid and Foscan®.

5.4.3 Clinical Studies of Photodynamic Therapy at Different Stages of Oral Cancer in Humans

Having worked out the histopathological effects of PDT on normal and neoplastic tissue, we were then able to investigate the treatment of various stages of oral cancer from dysplasia to advanced disease. We used three photosensitisers and tried to identify the most appropriate treatment for each disease stage.

All aspects of these clinical studies were under my direct care and supervision and carried out at University College London Hospitals NHS Trust.

Chapter 6: Animal Studies to Investigate the Photodynamic Therapy Effect on Normal Tissues

6.1 Introduction

Despite continued attempts to develop photosensitisers with improved selectivity, the concentration difference between normal and tumour tissue is still not sufficient to allow for effective tumour killing without some injury to surrounding normal tissue. It is therefore vital to determine the precise effects that photodynamic therapy will have on surrounding tissues, and to estimate their ability to recover over time (Bown 1990).

This is particularly important in the treatment of oral cavity cancers, where major blood vessels run in close proximity to the tumour and where tumours may be adjacent to, or may even have invaded, bone. It is also important to understand PDT effects on mucosa, muscle, nerves and salivary glands. A series of animal experiments were undertaken to look at some of these questions. The work in this section was co-supervised by me at the National Medical Laser Centre – the work on blood vessels was carried out by Mr W E Grant out between 1993 and 1994, before we had access to Foscan® and the bone study by Dr FMK Fan between 1994 and 1996.

6.2 PDT Effects on Blood Vessels

It is obviously important that we understand the effect of PDT on blood vessels in the head and neck. There are a number of large vessels whose rupture results in rapid death. The most obvious of these are the carotid arteries.

Two cases of fatal haemorrhage of the carotid artery 24 and 72 hours after photodynamic therapy have been reported in the literature (Schuller et al 1985, Gluckman 1991a). It is unclear if these were due entirely to the PDT effect or to direct tumour invasion of the artery.

Occlusion of the microvasculature is generally thought to be part of the mechanism of photodynamic therapy necrosis (see 4.1.1) but this conclusion

was reached through the study of small vessels such as arterioles, venules and capillaries. Small vessels with relatively little mural supporting connective tissue may be more likely to undergo occlusion or haemorrhage than larger arteries which have more smooth muscle, collagen and elastin components.

In order to evaluate the PDT effects on large vessels, two studies were undertaken, the first on the rat femoral artery and the second on the rabbit carotid artery.

6.2.1 PDT Effects on the Rat Femoral Artery

The response of larger vessels to photodynamic therapy was assessed in the rat femoral artery using ALA and disulphonated aluminium phthalocyanine: these two photosensitisers were selected for study due to the limited light penetration, inadequate tumour selectivity and residual photosensitivity of Photofrin®.

6.2.1.1 Materials and Methods

The normal femoral artery of the rat was chosen for study. This is a small muscular artery of 0.2 to 0.4mm in diameter, which consists of an intima with endothelial cells lying on a basement membrane, bounded by an inner elastic lamina, a media predominantly composed of smooth muscle cells, and an outer adventitial connective tissue layer.

Preliminary pharmacokinetic studies were undertaken to assess sensitiser distribution in the individual arterial layers and adjacent structures in order to establish the most appropriate timing of PDT. These studies showed that the fluorescence distribution pattern of phthalocyanine- and ALA-induced protoporphyrin IX was similar for both drugs. Maximal arterial fluorescence was detected in the intimal layer of each artery and similar fluorescence was seen in the adjacent thin-walled vein. This fluorescence was attributed to retention of circulating sensitiser by the endothelial cells in the case of the phthalocyanine and to uptake and metabolism of ALA to protoporphyrin IX, in the case of ALA-sensitised animals. Peak fluorescence was detected at 1 hour following

sensitisation with disulphonated aluminium phthalocyanine and at 3 hours with ALA sensitisation. The fluorescence ratio between arterial intima and media reached a maximum of 5:1 for disulphonated aluminium phthalocyanine and 3:1 for ALA at the same time as peak fluorescence was detected (1 and 3 hours respectively).

A second series of young adult female Wistar rats, were sensitised with ALA (200mg/kg) or disulphonated aluminium phthalocyanine (5mg/kg) and then treated at times corresponding to peak fluorescence (1 hour following sensitisation with disulphonated aluminium phthalocyanine, and 3 hours following ALA sensitisation). The rats were anaesthetised with Hypnorm and Diazepam, and a groin incision made on one side to expose the femoral neurovascular bundle, care being taken to avoid any surgical manipulation of the vessels which might result in damage to the delicate endothelium.

1cm segments of the femoral artery were exposed to laser light delivered by a copper vapour pumped tunable dye laser. Irradiation at 630nm was used for the ALA group as in previous studies from this unit (Bedwell et al 1992) although the *in vivo* excitation efficiency is slightly higher at 635nm; 675nm was used for the phthalocyanine group, which is in accord with the *in vivo* action spectrum (Cubeddu et al 1992).

In each drug group, eight rats were treated per time point, four at 100J/cm² and four at 250J/cm² delivered light using a surface illumination technique over a 1cm² area with a 400µm fused-silica optical fibre and a microlens attachment to ensure even light distribution. Power density was kept below 150mW/cm² to avoid thermal injury. This was confirmed in selected experiments using a copper-constantan thermocouple (Jenway, model 7905) placed immediately below the irradiated muscle surface to monitor temperature changes during the treatment; no rise in temperature was detected over the treatment times for either wavelength or light exposure. Following photodynamic therapy, the mid-point of the 1cm treated segment was marked by a 5:0 silk suture placed in the muscle at a distance from the treated zone, and the incisions closed. In the early phase of the investigation some rats removed their own sutures with wound dehiscence and sepsis; these animals were excluded from the study and

the procedure repeated in other animals using a transverse groin incision and closure, which did not suffer the same fate.

Animals were sacrificed at 3, 7, 14, 28, 84 and 168 days (6 months), the legs were severed the skin was removed, and fixed in 10% buffered formalin for three days. The legs were then decalcified and two or more sections were taken transversely through the mid-point of the treated segment. Sections were stained with haematoxylin and eosin (H&E), and the treated vessels identified and photographed at x40 using a Zeiss photomicroscope. Selected specimens were stained with elastin van Gieson for collagen and elastin, and Martius red scarlet blue for fibrin.

Three groups of control animals were studied at three days and one week: those that received light only at 250J/cm² at 630nm (two animals) and 675nm (two animals) and surgical exposure only (two animals). The contra-lateral non-treated leg was sampled in two animals from each treatment group at each time point up to six months and these acted as drug-only controls.

The luminal cross-sectional areas of the arteries were then measured on standard photomicrographs using a computer-aided image analysis system (Quantimet, Q520, Cambridge Instruments UK). Mean values were determined for each treatment group and compared with contra-lateral non-treated legs. This enabled a plot of cross-sectional area versus time to be constructed. Results were subjected to statistical analysis using Student's *t*-test.

6.2.2 Results

6.2.2.1 Nature of Injury

The arteries in control animals sacrificed at 3 and 7 days were patent and showed normal endothelium and normal media, indicating that light alone, surgical exposure alone or drug administration alone did not cause thermal or other significant injury. Subsequently contra-lateral non-treated arteries were used as controls for morphological comparison as these were matched for growth in the animals. No macroscopic change in the arterial configuration was

observed at any time. No arteries underwent occlusion, thrombosis, haemorrhage or rupture, or aneurysmal dilation, and all remained patent.

PDT-treated arteries in each drug group and at both light doses demonstrated a similar early response to PDT injury. Loss of endothelium and preservation of an intact inner elastic lamina (IEL) was characteristic. In spite of the loss of endothelium, no thrombus formation could be detected.

A striking feature was replacement of the entire smooth muscle cell population of the media by an homogeneous eosinophilic layer. No smooth muscle nuclei could be detected on light microscopy (see Figures 6.1a and d). There was complete loss of muscle tone with wide dilation of the artery and smooth configuration of the inner elastic lamina, a structure that was observed in all controls to be corrugated owing to the smooth muscle tone of the vessel. PDT thus appeared to render the vessel an acellular conduit.

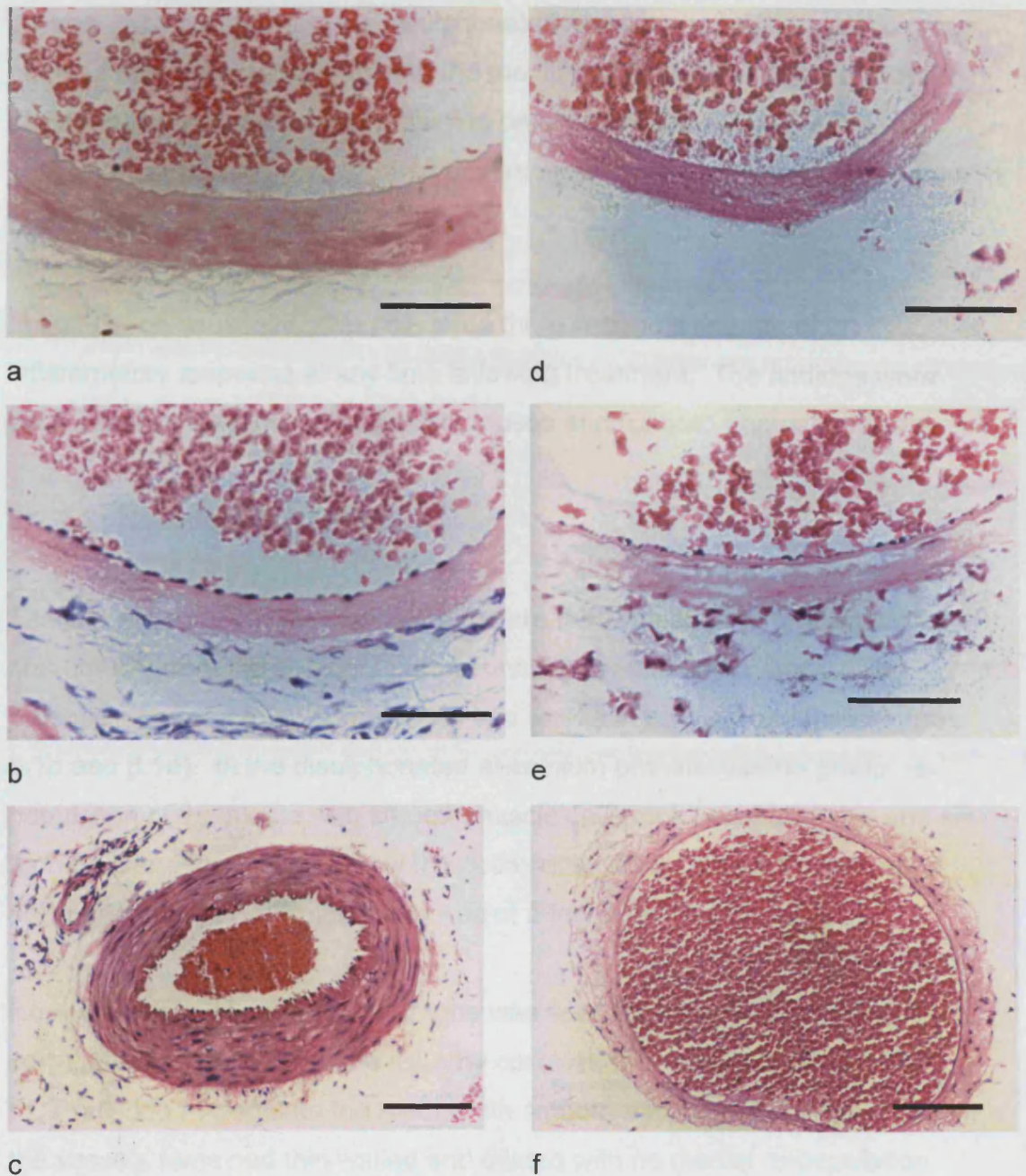


Fig 6.1: PDT effects on rat femoral artery

Haematoxylin and eosin light micrographs of arteries treated with PDT at times up to six months. Photomicrographs on the left show arteries sensitised with 5mg/kg disulphonated aluminium phthalocyanine and treated with 100J/cm² laser light at 675nm, sacrificed at (a) 3 days (b) 14 days and (c) 168 days. On the right are shown arteries sensitised with 200mg/kg ALA and treated with 100J/cm² laser light at 630nm (d,e and, f) again showing the appearance at 3, 14 and 168 days respectively. At 3 days in both groups there is complete cellular depletion throughout the vessel wall, and the intact inner elastic lamina is clearly seen. The endothelium has regenerated by 14 days in both drug

groups. At 168 days only the disulphonated aluminium phthalocyanine group showed complete re-population of the media with functional smooth muscle cells, the ALA-treated artery remaining persistently dilated, with no medial smooth muscle cells. Scale bar in bottom right corner represents 50 μ m for a, b, d, e (x100) and 100 μ m for c and f (x40)

In spite of obvious extensive cell death there was no evidence of an inflammatory response at any time following treatment. The findings were similar for both the low and high drug doses and for both photosensitisers.

6.2.2.2 Healing and Recovery

The lost endothelium was the first structure that was seen to regenerate, presumably from the adjacent normal untreated ends of the artery. This process appeared to be complete by two weeks in both groups (see Figures 6.1b and 6.1e). In the disulphonated aluminium phthalocyanine group, re-population of the media with smooth muscle cells took between three and six months (see Figure 6.1c). Only the occasional vessel showed evidence of slight neo-intimal hyperplasia that was of a few cells' thickness.

Normal contractility and vascular tone was seen with the return of the corrugated appearance of the IEL. By contrast, the vessels treated with ALA-PDT failed to re-populate the media with smooth muscle cells. After six months, the vessels remained thin walled and dilated with no medial re-population (Figure 6.1g). The IEL remained smooth and straight, reflecting this finding. All vessels remained patent with no evidence of thrombosis at any stage.

The cross-sectional area of the treated vessels was significantly greater in all treatment groups when compared with controls up to four weeks. This corresponded to the observed loss of media muscle tone of the vessels. By six months the vessels had recovered in the disulphonated aluminium phthalocyanine group but remained significantly dilated in the ALA-treated group (Table 1). However, no macroscopic aneurysmal dilation was observed.

Time (days)	Controls	AlS ₂ Pc 100J/cm ²	AlS ₂ Pc 250J/cm ²	ALA 100J/cm ²	ALA 250J/cm ²
3	0.011(±0.003)	0.062(±0.001)	0.056(±0.004)	0.071(±0.007)	0.065(±0.010)
7	0.008(±0.002)	0.056(±0.002)	0.050(±0.001)	0.064(±0.008)	0.074(±0.005)
14	0.012(±0.003)	0.0467(±0.003)	0.054(±0.003)	0.043(±0.005)	0.064(±0.020)
28	0.009(±0.003)	0.058(±0.016*)	0.028(±0.008) NS	0.060(±0.005)	0.066(±0.005*)
84	0.011(±0.003)	0.042(±0.011*)	0.027(±0.009) NS	0.085(±0.005)	0.087(±0.003)
168	0.011(±0.003)	0.009(±0.001) NS	0.016(±0.003) NS	0.096(±0.002)	0.091(±0.004)

Table 6.1: Mean (± standard error mean) of arterial luminal cross-sectional areas

Values are in mm² for cross-sectional areas at each time point. Figures are means, with standard error of the mean given in brackets. Control values showed no significant difference between drug groups and therefore mean and standard error of mean values are presented for all controls at each time point. NS = not statistically different from control values. * = significant at the P<0.05 level; remainder significant at P<0.001 level.

6.2.3 Discussion

The observation that there was no difference in histological response between the 100J/cm² and the 250J/cm² light doses in either of the drug groups indicates that both light doses are above a threshold for injury, and that above this threshold, damage does not appear to increase with light dose. The similarity of response in the early phase following PDT in both the ALA and disulphonated aluminium phthalocyanine groups indicates that a similar photodynamic mechanism is responsible for the injury in each group.

The patency of all treated vessels, and the lack of evidence of thrombosis in spite of elimination of the endothelium, is encouraging and suggests that occlusion of major blood vessels due to PDT is unlikely to be a problem. Furthermore, the vessel walls all remained intact, suggesting a resistance of the injured walls to haemorrhage or disintegration under physiological stresses in

spite of full-thickness cell death. It has been postulated that there was preservation of an intact inner elastic lamina, as well as the preservation of normal adventitial collagen. This indicates that these acellular supportive elements are not denatured by PDT, and contribute to the preservation of the mechanical integrity of the vessels.

The absence of mural inflammation in the presence of the apparent extensive cell death, together with the persistent function of the vessels, suggests that typical cell necrosis may not be taking place, and that the features might be consistent with a form of programmed cell death, or apoptosis (see Section 4.1.1).

It is well established that PDT in tumour therapy brings about necrosis at least in part by virtue of its action on the microvasculature and endothelial cells have also been shown to be highly sensitive to PDT injury (see Section 4.1.1). Certainly the endothelium in this study convincingly demonstrated maximal fluorescence for both drugs used, suggesting the likelihood of high susceptibility to photodynamic injury. This is in contrast to the work of LaMuraglia et al (1993) who found fluorescence in the full thickness of the arterial wall.

While relatively low levels of fluorescence were seen in the smooth muscle of the vascular media, treatment groups showed a dramatic response to light exposure. Light-only and drug-only groups showed no medial injury. Therefore, in spite of low fluorescence detection, these cells appear to be highly susceptible to photodynamic injury.

Blood vessels of this size are oxygenated by circulating blood in the lumen and do not have vasa vasorum, indicating that a direct phototoxic effect was responsible rather than the injury being secondary to ischaemia due to microvascular shutdown.

In contrast to Suzuki et al (1987) who found that endothelial cell regeneration in rats occurred within five days, in this study, the endothelium, although showing some evidence of regeneration at one week, was not complete until two weeks. Chevetton et al (1992) reporting on skeletal muscle injury after PDT using a

variety of sensitisers noted loss of endothelium, and intravascular thrombosis in arterioles. An eosinophilic necrosis of arteriolar smooth muscle was also described and the changes were reminiscent of those seen in acute hypertension.

These findings are similar to our observations with the exception that there was no evidence of thrombosis in the larger arteries examined in this study.

Our study also looked at the long-term effects of PDT on normal arteries at follow-up times of up to six months in the treated groups. Re-population of the cell-depleted media took from three to six months in the disulphonated aluminium phthalocyanine groups. This re-population was associated with the observation that occasional vessels showed intimal hyperplasia of only one or two layers' thickness and did not appear to have any degree of stenosis. As no significant intimal hyperplasia was demonstrated by six months in the present study group, long after endothelial regeneration was completed, we concluded it was therefore unlikely to develop.

In the ALA group, medial re-population had not occurred by six months, and was thus similarly felt unlikely to occur at all. This finding was difficult to explain and suggests a local, perhaps biochemical, inhibitory effect resulting from a difference in the nature of the injury not detected by the morphological investigations carried out. The effect on the treated arteries in this group was to transform the artery into a wider bore non-contractile vessel with an adventitia and an intima but no functional media. While vein-grafted stenoses in man tend to undergo arterialisation by migration of smooth muscle cells from adjacent arterial ends (Dilley et al 1988), this had not taken place by six months. This may have clinical relevance in that vessels treated following sensitisation with ALA may be even less likely to develop intimal hyperplasia. A possible outcome may be long-term weakness of the vessels and a tendency to aneurysmal dilation, however this was not observed macroscopically in this study.

In spite of obvious extensive injury both to endothelial cells and medial smooth muscle cells, neither stenosis nor intimal proliferation of smooth muscle cells

was observed in this study. This may reflect the total nature of the injury to the vessel walls studied leaving behind no residual SMCs in the treated segments.

A possible explanation for the lack of intimal hyperplasia is that endothelial regeneration occurs at a much faster rate than medial re-population, and once completed acts to prevent migration of proliferating SMC. The endothelium in balloon-denuded vessels regenerates from the ends of the denuded segment (Clowes et al 1983) and it is likely that a similar process occurs in PDT-injured vessels for both endothelial cells and smooth muscle cells.

The cross-sectional areas looked at in this study illustrate the effects of PDT injury to the vessel wall, with significant dilation being observed compared to controls at up to 14 days in all treatment groups. They further reflect the observed medial re-population in the disulphonated aluminium phthalocyanine group at six months, confirming that functional recovery takes place in this group and that the SMCs are phenotypically contractile.

The persistent increase in cross-sectional area in the ALA group even at six months illustrates the failure to re-populate the media with functional SMCs. A criticism of the method is that the harvested vessels were not perfusion fixed, and therefore may not exhibit their *in vivo* configuration. However, consistent results were obtained, and clearly demonstrate a statistically significant difference in response to PDT between controls and ALA- and disulphonated aluminium phthalocyanine-treated groups.

As has been discussed in Chapter 4, PDT tumour necrosis is brought about at least in part by microvascular collapse with thrombosis and haemorrhage. It would appear from this study that larger vessels with sufficient supporting mural connective tissue elements are resistant to such collapse. It remains to be determined which size of vessel represents the cut-off point for haemorrhage and occlusion.

Furthermore, should tumour fluorescence profiles differ significantly from those demonstrated in large blood vessels, it may be possible to identify times at which tumour PDT would not result in significant vascular injury.

6.3 PDT Effect on Rabbit Arteries

Following this, a second study using the carotid artery in rabbits was carried out to determine whether the photodynamic effect weakened the walls of larger vessels, thereby increasing the risk of haemorrhage through loss of mechanical integrity and rupture of the artery wall. In this study, the strength of treated vessel walls was assessed by intra-luminal hydrostatic distention to bursting point.

6.3.1 Materials and Methods

New Zealand white rabbits, under fentanyl and fluanisone sedation, were injected with either ALA at 200mg/kg (Sigma Chemicals) in phosphate buffered saline or disulphonated aluminium phthalocyanine (in solution as prepared by the Department of Chemistry, Imperial College London) at 1mg/kg via an ear vein.

Photodynamic therapy was applied by anaesthetising the animals at appropriate periods and gently dissecting the common carotid arteries on both sides. The arteries were optically isolated from surrounding structures by placing a piece of foil-lined opaque card between them and the underlying tissues. A 1cm length of each artery was exposed to a 100J/cm² dose of light from a copper-vapour pumped dye laser. A light dose of this order corresponds to a typical tumouricidal dose (Grant et al 1993a). Light at 630nm was used for ALA-treated rabbits, and light at 675nm was used for disulphonated aluminium phthalocyanine-treated rabbits. These wavelengths correspond to peaks in the absorption spectra of protoporphyrin IX and disulphonated aluminium phthalocyanine respectively. The surface was illuminated using a 400µm fused silica optical fibre with a microlens attachment.

The rabbits underwent PDT three hours after receiving ALA and one hour after receiving disulphonated aluminium phthalocyanine. These times were chosen to correspond with peak levels of sensitiser as determined by fluorescence photometry studies (Loh et al 1992, Grant et al 1993b [Chapter 10], Bedwell et al 1992, Grant et al 1994 [pd study referred to in chapter 6]).

The treated segments were marked by placing two 5:0 silk sutures in the adjacent fascia of the carotid sheath. After PDT, the incisions were closed, and the animals were revived and returned to their cages. No animals suffered any obvious adverse effect from the therapy.

To confirm that PDT produced vascular injury, four rabbits (two from each drug group) were sacrificed at three days. Their carotid arteries were removed and were fixed for haematoxylin and eosin (H&E) and elastin van Gieson staining. Transverse sections were taken from the treated segments and from adjacent segments that were not exposed to light; the latter served as the controls with 'drug plus dissection but no light'. Two other rabbits that received the same dose of light but without prior photosensitisation served as the 'light only' controls; these animals were also sacrificed at three days.

A further 18 rabbits underwent identical treatments: nine with ALA and nine with disulphonated aluminium phthalocyanine. Three rabbits from each group were sacrificed on day three, three more from each group were sacrificed on day seven, and the last three from each group were sacrificed on day 21.

After sacrifice, the neck incisions were re-opened, the carotid arteries were re-excised, and the treated segments were identified. A blunted 18-gauge butterfly needle was inserted into each of the treated sections, and the section was then gently irrigated with normal saline. Next the needle was isolated in half of the treated section using 4:0 silk ligatures. The needle was connected to a three-way tap coupled to a pressure transducer and to the output of a water-filled pressurised vessel. The pressure transducer was connected to a power supply, a chart recorder, and a digital voltmeter. The input to the pressure vessel was connected to a compressed-air cylinder through a manual pressure regulator capable of delivering up to 16 bar (12,000mmHg) see Figure 6.2. This enabled pressure to be gradually increased until the isolated vessel segment ruptured, which usually happened after several seconds.

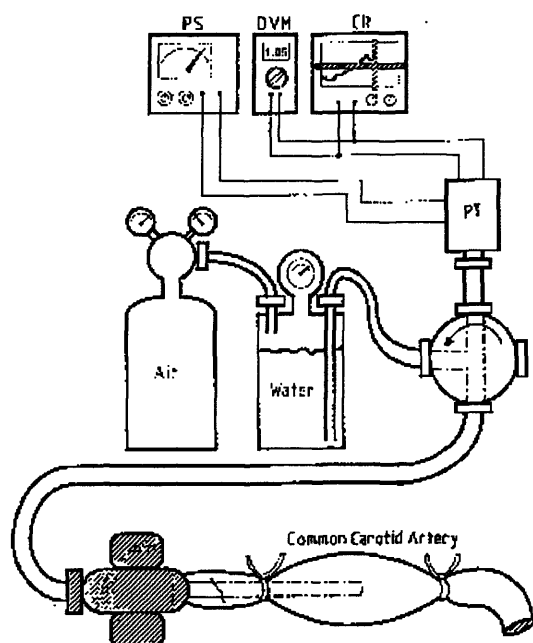


Fig 6.2: Apparatus and method of isolation and hydrostatic distention used in treated segments of carotid artery (PS = power supply; DVM = digital voltmeter; CR = chart recorder; PT = pressure transducer)

The procedure was repeated for the other half of the treated segment, as well as for the proximal and distal normal segments of the same artery, which served as controls. The procedure was also repeated on the contra-lateral treated and untreated segments of carotid artery. Consequently, four treatment values and four control values were obtained for each rabbit.

Mean values for treated and control segments were calculated. Bursting pressure measurements from each treatment group were compared with the measurements from the controls and were statistically analysed using Student's *t* test.

6.3.2 Results

All rabbits tolerated the treatment satisfactorily and showed no ill effects in the post-operative period. When the treated and control arteries were exposed, no evidence of dilatation, aneurysm formation, or obvious diminished blood flow could be determined. Macroscopically, the treated sections appeared a little paler than the adjacent untreated segments.

The treated arteries sampled at three days all showed evidence of characteristic PDT injury. The same injury was observed in both drug groups on histologic examination after H&E staining. The injury consisted of loss of endothelium and evidence of complete cell death throughout the media and adventitia. In contrast, all sections from the control arteries showed normal arterial features, with an intact and cellular intima, media, and adventitia. Histological examination following elastin and collagen van Gieson staining demonstrated no discernible difference between treated and control arteries. Both showed normal configuration of collagen in the media and the adventitia, as well as an intact inner elastic lamina and intact medial elastic laminae (Figure 6.3).

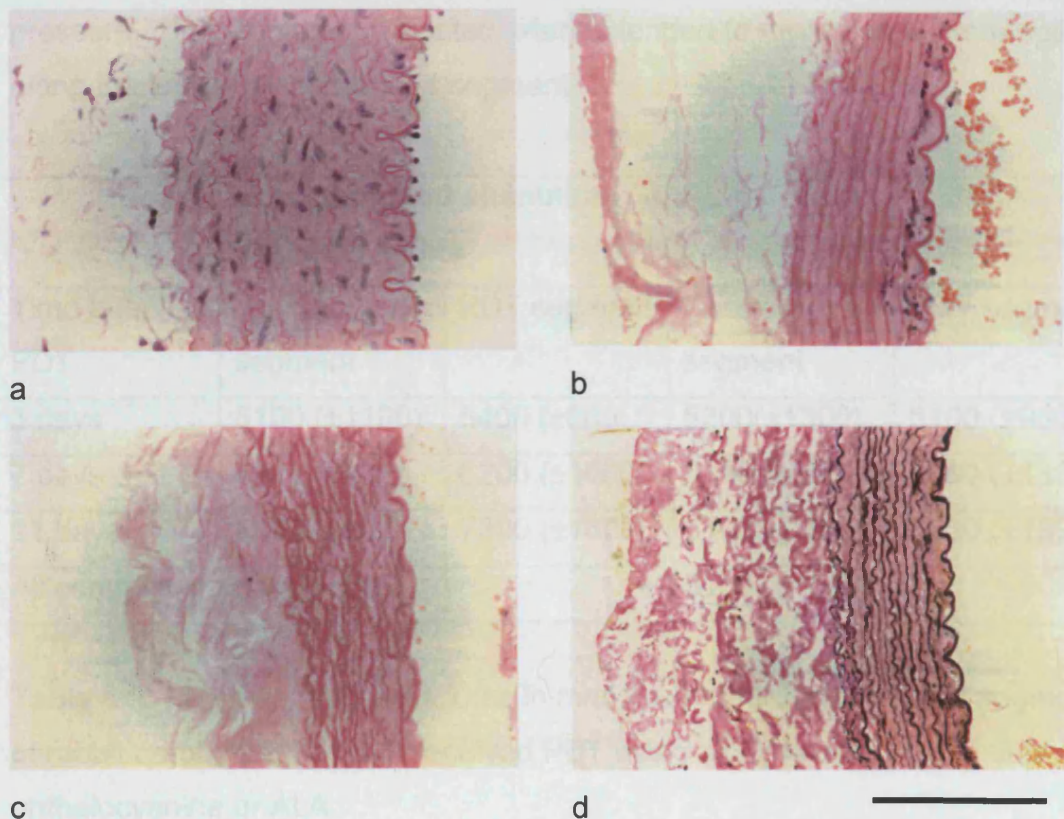


Fig 6.3: Photomicrographs of rabbit carotid arteries treated with PDT (a) H&E-stained normal rabbit carotid artery. (b) carotid artery three days after photodynamic therapy using ALA. Sub-intimal pyknotic nuclei and nuclear debris are seen in the photomicrograph. (c) carotid artery three days after PDT using disulphonated aluminium phthalocyanine. (d) Van Gieson-stained vessel after treatment with PDT using ALA. The inner elastic lamina and medial elastic laminae demonstrate a normal configuration. A similar staining pattern (not shown) was observed following PDT with disulphonated aluminium

phthalocyanine (original magnification x 60 Bar at bottom right represents 100 μ m).

Control arterial segments (drug plus dissection but no light) were found to burst at a mean of 5100mmHg (SD= \pm 1100mmHg) in the phthalocyanine group and 5200mmHg (SD = \pm 1300 mmHg) in the ALA group. The overall mean was 5100mmHg (SD= \pm 990). At three days, there was no significant difference in the hydrostatic pressure required to burst the treated or control segments. At seven and 21 days, however, considerably greater pressure was required to burst the treated segments than the control segments (Table 6.2). The pressure required to burst all vessels was 50 to 70 times the arterial blood pressure. Both control and treated arteries tended to rupture in a linear split along the length of the isolated segment.

	Disulphonated aluminium phthalocyanine		ALA	
Time after PDT	Control segment	PDT segment	Control segment	PDT segment
3 days	5100 (\pm 1100)	5400 (\pm 800)	5200(\pm 1300)	5100 (\pm 900)
7 days	4900 (\pm 900)	6200 (\pm 1600)	5100 (\pm 800)	6800 (\pm 1300)
21 days	5500 (\pm 800)	7300 (\pm 1600)	5100 (\pm 1000)	6800 (\pm 1800)
All controls	5200 (\pm 900)		5100 (\pm 1000)	

Table 6.2: Mean bursting pressures in mmHg for control and treated segments of rabbit carotid arteries that received PDT using disulphonated aluminium phthalocyanine or ALA

6.3.3 Discussion

The expected histologic effects of PDT using ALA and disulphonated aluminium phthalocyanine as photosensitisers was observed. Endothelial cells were lost from the intima, and cellular structures were completely lost in the media. Similar findings were observed in a previous study (Section 6.2) and by other investigators (Ortu et al 1992).

All treated segments demonstrated that full circumferential injury was caused by the chosen treatment parameters. The control 'drug plus dissection but no light' groups and the 'light only' groups demonstrated preservation of normal cellular intima, media and adventitia. The fact that bilateral PDT of the common carotid artery did not result in thrombotic occlusion is demonstrated by the failure of any treated rabbit to show signs of stroke. Furthermore, no histologic evidence of mural thrombosis was found.

This study demonstrates that, despite the full-thickness cell death PDT induces in the vessel wall, no weakening or loss of mechanical integrity occurs. It is not unreasonable to suppose that an inflammatory response might be observed with such extensive necrosis and that the release of inflammatory mediators might contribute to weakening of the vessel wall. It is possible that PDT in this situation provokes instead a form of programmed cell death, similar to apoptosis, in which no inflammatory response occurs (see 4.1.1). This would help to explain the preservation of the mechanical integrity of the vessels in this study. Furthermore, the fact that PDT does not result in degradation or denaturation of collagen and elastin probably contributes to the preservation of function (Barr 1987a).

Other studies of PDT-treated rat colons (Barr 1987b) and rat tracheas (Smith 1993) have shown preservation of mural collagen and no reduction in the hydrostatic pressure required to rupture the treated organ. One possible contributory factor is that through singlet oxygen reactions, PDT brings about the cross-linking of collagen, which increases its resistance to deformation (Verweji et al 1981). The increase in pressure required to burst the vessels at 7 and 21 days might be explained further by the re-population of the media and to a lesser extent the intima, by proliferating smooth muscle cells, resulting in a minor degree of neointimal hyperplasia.

6.4 Conclusion: PDT Effect on Arteries

These two studies demonstrated that arteries treated with photodynamic therapy using disulphonated aluminium phthalocyanine and ALA remain patent without rupture, haemorrhage or thrombotic occlusion. Furthermore they

provided reassurance that, despite full-thickness vascular mural cell death following PDT, mechanical weakening of the treated vessel walls does not occur.

Non-cellular structural elements such as collagen and elastin are preserved and this, coupled with the lack of inflammatory response in the arterial wall in spite of extensive cell death, indicate that mechanical integrity is preserved. Long-term patency was demonstrated with minimal risk of development of intimal hyperplasia.

These findings indicate that arteries exposed to PDT during tumour therapy are unlikely to be at risk, provided there is no direct invasion of the artery wall by tumour. This has significance for the use of PDT in tumours located near major vessels, such as may occur in the head and neck, and for the intra-operative adjunctive use of PDT to ablate microscopic residual disease. However, while one must be cautious about the extrapolation of these results to other photosensitisers, recent work by Kübler using Foscan® had similar results. So this effect probably translates to most currently available photosensitising agents.

6.5 PDT Effect on Bone

6.5.1 Bone

Studies on the rabbit mandible (Meyer et al 1991) using disulphonated aluminium phthalocyanine suggest that bone is resistant to PDT treatment. However, these studies did not look at the three sensitizers that are currently used in clinical practice: 5-aminolaevulinic acid, Photofrin® and mTHPC. The study below was designed to address this issue and evaluate PDT effects on normal bone.

6.5.2 Materials and Methods

Fifty-eight female New Zealand White rabbits were used in two series of experiments: the first to determine the effect of PDT on the bone of the tooth

socket and the second to verify that the parameters used were capable of producing necrosis.

Three photosensitisers were used: Photofrin®, 5-aminolaevulinic acid and mTHPC. Photofrin® was reconstituted using 5% dextrose, to make a 2.5mg/ml solution for intravenous injection at a dose of 3mg/kg. ALA hydrochloride in 98% pure crystalline form was dissolved in phosphate buffered saline, giving a 100mg/ml solution for oral administration at a dose of 400mg/kg. The Foscan® crystals were dissolved in a mixture of polyethylene glycol, water and ethanol to produce a 5mg/ml concentration which was administered at a dose of 0.3mg/kg body weight IV.

The total dose of 400mg/kg of ALA was delivered in three equal fractions, with a one hour interval between each administration, consistent with our current clinical protocol (Fan et al 1996). The fractionated ALA dose was chosen on the basis of established pharmacokinetic principles (Regula et al 1995).

6.5.3 PDT on Normal Bone

Forty-eight rabbits were divided into six groups as shown in Table 6.3. The four treatment groups consisted of two Photofrin® groups, an ALA and a Foscan® group and two 'light only' control groups.

	Photofrin ® 1 hour	Photofrin ® 48 hours	ALA 2.5 and 4 hours	mTHPC 72 hours	Light alone	Light alone
Dose/route	3 mg/kg IV	3 mg/kg IV	400 mg/kg oral	0.3 mg/kg IV	N/a	N/a
Wavelength	630 nm	630 nm	630 nm	650 nm	630 nm	650 nm
Drug/laser interval, h	1	48	2.5 and 4	72	N/a	N/a
Light dose	100 J	100 J	2 x 100 J	100 J	100 J	100 J
Number of rabbits	9	9	9	9	6	6
Time to sacrifice, days	3 x 3 3 x 10 3 x 21	3 x 3 3 x 10 3 x 21	3 x 3 3 x 10 3 x 21	3 x 3 3 x 10 3 x 21	2 x 3 2 x 10 2 x 21	2 x 3 2 x 10 2 x 21

Table 6.3: Treatment protocol for drug and light administration to rabbit tooth socket

The rabbit model used in these experiments was as described by Meyer et al (1991) (also see Chapter 5) but with some refinements. A cylindrical diffusing fibre was used instead of a bare cut fibre to enable more uniform illumination of the entire tooth socket.

To simulate the infiltration of tumour into bone, all rabbits in the first part of the study had the lower mandibular incisor removed. If one considers the void to represent tumour, then PDT of that area will result in the same normal tissues being exposed to PDT. The ipsilateral maxillary incisor was also extracted to act as a control for the effect of the photosensitiser and trauma of the extraction process. Each rabbit therefore yielded two samples of tissue for analysis. Ideally controls should have been carried out on the contralateral mandibular incisor, but this could have led to feeding problems. The different experimental groups are summarised in Table 6.4.

	Photofrin®		Photofrin®		ALA		MTHPC		Light alone	Light alone	Extn alone
Site of extraction	Man	Max	Man	Max	Man	Max	Man	Max	Man	Man	Max
Irradiation	Yes	No	Yes	No	Yes	No	Yes	No	Yes, 630nm	Yes, 650nm	No
Drug/light interval, h	1		48		2.5 & 4		72				
Number of rabbits	9	9	9	9	9	9	9	9	6	6	6

Table 6.4: PDT and control groups, treatment schedule

Oral delivery of ALA required the rabbits to be sedated prior to administration. In all other cases, removal of the tooth and subsequent laser irradiation were carried out with the animal anaesthetised using 2mg/kg diazepam administered subcutaneously and maintained IV, and 0.3ml/kg Hypnorm administered intramuscularly.

The light source was a pulsed 12kHz copper vapour pumped dye laser (Cu25/DL10, Oxford Lasers Ltd, Abingdon) and the output was focused into a 0.4mm core diameter optical fibre with a 1cm cylindrically diffusing tip (PDT Systems, Santa Barbara, Ca). The fibre was passed into the base of the tooth socket, allowing near uniform irradiation of the whole of the defect (Figure 6.4). The power output was selected in order to supply 100mW/cm² at the fibre tip, with an exposure time of 1000 seconds to achieve a total dose of 100J/cm².

Light administration was carried out at 1 and 48 hours post-sensitisation with Photofrin®, and 72 hours post-sensitisation with mTHPC. A fractionated irradiation regimen was used with ALA, with a 75 minute break or dark period halfway through illumination (100J/cm² at t = 2.5 hours and 100J/cm² at t = 4 hours).

The maxillae and mandibles of the rabbits which had received PDT to the tooth sockets were decalcified using trichloroacetic acid and longitudinal sections were taken through the tooth socket. Tissue sections were processed in

paraffin. Haematoxylin and eosin (H&E) stained sections were examined for damage or necrosis to the normal bone in addition to the various stages of bone healing. The presence of the following were assessed: haematoma, granulation tissue, inflammatory response, osteoid and woven bone formation. Woven bone formation was scored semi-quantitatively on a scale of 0 to 3.

6.5.4 Results

6.5.4.1 Bone Healing

There was no significant difference seen between the amount of woven bone in any of the control groups (Table 6.5). In each of the drug only groups there was woven bone formation at the edge of the socket by 3 days, which filled the socket by 10 days and this was similar to the extraction alone group. In the light only control group using light at 630nm, woven bone formation was identical to the three drug control groups, however, that at 650nm was slightly slower.

With all three photosensitisers, woven bone formation was more rapid in the drug only control groups, but by day 21 there was a similar degree of woven bone formation in control and treated groups. When healing of all four PDT groups were compared, those that had been sensitised with Foscan® had more rapid complete bone healing than those which received Photofrin® or ALA (Table 6.5). The most important finding was the absence of any necrotic bone or non-viable osteoblasts.

Group	Day 3	Day 10	Day 21
Photofrin PDT (1h)	+	+++	+++
	0	++	++
	0	++	+++
Photofrin PDT (48h)	0	++	+++
	0	++	+++
	0	+++	++
Photofrin drug control	+	+++	+++
	+	+++	+++
	+	++	+++
ALA PDT	++	++	+++
	++	++	+++
	+	+++	+++
ALA drug control	+	+	+++
	+	+++	+++
	0	+++	+++
mTHPC PDT	0	+++	+++
	0	+++	+++
	0	+++	+++
mTHPC drug control	+	+++	+++
	+	++	+++
	0	+++	+++
Light 630nm	+	++	+++
	+	++	+++
Light 652nm	0	++	+++
	0	++	+++
Extraction only	+	++	+++
	+	++	+++

Table 6.5: Woven bone score of individual maxillary and mandibular specimens

Key: 0: absence or minimal presence of woven bone along the socket margin (score = 0)

+: presence of woven bone along the socket margin (score = 1)

++: socket half filled with new bone (score = 2)

+++ : socket at least $\frac{3}{4}$ filled with bone (score = 3)

Osteoid formation was present by 3 days in most specimens and granulation tissue was also most abundant. No muscle scarring was evident and no damage was seen to the salivary tissue in any group.

6.5.5 Discussion

These studies showed that treatment of the tooth socket with PDT, under treatment conditions that would bring about tumour necrosis, did not cause

bone necrosis and only brought about a minor delay in the healing process to the bone after tooth extraction. PDT appears to retard bone healing in the early stages, but does not affect the long-term outcome. At the concentrations used, none of the photosensitisers on their own had any adverse effects on the overall rate of bone healing and light alone at 630nm had no apparent effect.

6.6 PDT on Buccal Mucosa

6.6.1 Materials and Methods

The second series of experiments was carried out on both the left and right buccal mucosa of ten rabbits drawn from the sensitised groups. After sensitisation as described in the previous section the following treatment schedule was carried out:

	Photofrin® 1 hour	Photofrin® 48 hours	ALA 2.5 & 4hrs	mTHPC 72 hours	
Number of rabbits	2	2	2	2	2
Wavelength	630 nm	630 nm	630 nm	650 nm	650 nm
Energy density	125 J/cm ²	125 J/cm ²	2 x 125 J/cm ²	125 J/cm ²	31 J/cm ²

Table 6.6: PDT on buccal mucosa, treatment schedule

The same drug-light intervals as above were used (1 hour and 48 hours for Photofrin®, 2.5 and 4 hours for ALA and 72 hours for mTHPC table 6.5). The laser beam was focused onto a 0.4mm core diameter microlens fibre (QLT Phototherapeutics, New York, USA) allowing delivery of a uniform, 5mm diameter circular spot of light to the buccal mucosa with a power density of 127mW/cm². The higher energy density was used to match as closely as possible those delivered to the bone defect, as calculated from the size of the removed tooth (0.8cm²). The lower fluence was initially used with Foscan® to avoid extensive damage observed in clinical cases irradiated with 20J/cm², but these experiments were later repeated with the higher energy density to facilitate comparison. Three days following PDT, the animals were sacrificed

and the ulcers and a margin of normal surrounding buccal mucosa were excised.

Following PDT to the buccal mucosa, the ulcers and margin were excised and immediately fixed in neutral buffered formalin and processed to paraffin blocks. Five micrometer sections were cut and stained with H&E for comparative light microscopy studies. Due to variable amounts of sloughing amongst the different specimens, the depths of necrosis were gauged by the different tissue levels affected. The depth of necrosis was defined as the deepest point at which there was necrosis of a tissue layer.

6.6.2 Healing of Buccal Mucosa

Necrosis was present in all specimens. The depth of necrosis in all Photofrin® and Foscan® specimens was comparable, while the depth of damage with ALA was much more superficial. The low energy density used initially with the Foscan® rabbits ($31\text{J}/\text{cm}^2$) showed dramatic effects on soft tissue at a very low light dose. When the experiments were repeated at the higher dose of $125\text{J}/\text{cm}^2$, slightly greater depth of damage was observed. There was little difference in the Photofrin® sections using the different irradiation times.

Similar histological features were observed in the Photofrin® and Foscan® samples: necrosis extending well into the muscle layer and vascular damage with fibrinoid degeneration and thrombus formation. From these findings it seems likely that the cost of greater necrosis as seen with Photofrin® and Foscan® is greater tissue disruption which has the potential to cause scarring.

6.6.3 Discussion

PDT in the therapeutic range that would be of value to treat cancer is also very likely to cause mucosal damage. This is less likely to occur with ALA, where the effect is much more superficial. In terms of our understanding of mucosal healing following PDT, this study really didn't take our knowledge significantly further than the Meyer studies in 1991.

6.7 Conclusion

It is clear from these studies that PDT is safe in close proximity with blood vessels in the normal setting. It would be quite reasonable to speculate that in a clinical setting, where tumour was eroding the vessel wall, PDT would not be safe and might precipitate acute haemorrhage. However, as long as tumour is close but not eroding the arterial wall, treatment can be carried out safely. The main criticism of the experiments outlined above is that we did not look at bursting pressures of rabbit carotids using Foscan®. However Kübler repeated these experiments using mTHPC on rabbit carotid and femoral vessels and also looked at the vagus and femoral nerves. In a dose escalation study he found that at high drug doses (0.3mg/kg) and a short drug light interval (24hours) with a light dose of 20J/cm² there was oedema, some thrombosis and disruption of the endothelial layer. He also reported up to 75% demyelination, but importantly, neither caused any clinical distress and no vessel rupture was seen (Kübler et al 2003).

Bone treated with PDT does show some slightly impaired healing. It is not entirely clear what mechanism is involved, but microvascular damage with reduced blood supply to the healing site is possible. Fortunately, no long-term problems were seen with bone healing and this is obviously of great importance for any treatment in the oral cavity. In many ways, bone involvement with tumour might be a good target for PDT treatment. Radiotherapy is often difficult near bone because of the risk of osteoradionecrosis and surgical resection is either mutilating or requires tissue transfer reconstruction. This is obviously a great deal of treatment for the patient to undergo, especially bearing in mind the co-morbidity in this group of patients.

In conclusion, while it is important to be aware of potential problems with normal tissue healing after PDT, none of the animal work suggests insurmountable problems and a reasonable amount of safety data suggests PDT is safe to take forward into clinical practice.

Chapter 7: PDT Effects on Human Tissue using Three Photosensitisers: Photofrin®, ALA and mTHPC

7.1 Introduction

The aim of this series of studies was to determine the effects of PDT on normal and tumour tissue with the 3 commonly used sensitisers. The methodology varied slightly in each study, but the broad aim was to determine the depth of PDT effect and the histological changes in normal and malignant tissue. This was considered an essential step of understanding the PDT effect in humans and in a clinically relevant setting, so that possible treatment applications could be developed. All the studies here were performed on my patients in ethically approved studies for which I was principal investigator. Treatments were carried out by me or under my supervision by Mr Grant (Photofrin® and ALA) of Ms Fan (ALA and Foscan®). The Photofrin® work was performed in 1992-1994, the ALA work 1993 –1996 and the Foscan® work 1995 – 1998.

Photodynamic therapy has many applications but it is considered to be particularly suitable for local tumour destruction in the oral cavity as conventional treatment results either in tissue loss, dysfunction and mutilation (surgery) or may produce irreversible side effects (radiotherapy), see Chapter 3 for more detail.

Photodynamic therapy may be performed as an out-patient procedure and excellent healing has been reported following such treatment in animal models and pilot clinical trials (Grant et al 1993a, Gilson et al 1988, Barr et al 1987a, Meyer et al 1991). It has often been claimed that PDT can be used to achieve a selective tumour necrosis but although some agents such as the porphyrins can be used as tumour-localising agents in photodiagnosis due to the preferential concentration in tumour tissue (Monnier et al 1990), the effect is not sufficiently pronounced to allow tumour damage without injury to normal tissue with treatment parameters that are required for therapeutic effect. Where selectivity of effect is claimed this is often only through the accurate distribution of light, confined to the tumour area. Some apparent selectivity may also be seen if the illumination is less in the peripheral zone of the spot than centrally.

For each photosensitiser it is important to know the depth of necrosis with a given light dose in order to be certain of satisfactory tumour ablation. This highlights the importance of careful assessment of the depth of the tumour.

In these studies, the author aimed to assess the histological nature of PDT injury to normal and neoplastic tissue, evidence of selectivity, and the depth of injury for a given light and drug dose using Photofrin®, ALA and mTHPC.

7.2 PDT Effect on Human Tissue with Photofrin®

7.2.1 Materials and Methods

Following review by the ethics committee at UCLH NHS Trust, 11 patients with histologically proven T₁ squamous-cell carcinomas of the oral cavity who were scheduled for surgical excision were recruited. This was a treat and excise study and the sites treated are summarised in Table 7.1. It was planned to formally excise the lesions at a time when necrosis was apparent, but before the necrotic slough had chance to separate that is to say between 2 and 7 days. Only 1 patient had a delay of 19 days because he declined earlier surgery. All patients received Photofrin® (Quadra Logic Technologies, Vancouver, Canada) at 2mg/kg iv, 48 hours prior to light exposure of tumours and a surrounding cuff of normal tissue. Exposure was with 50J/cm² laser light at a wavelength of 630nm with a power density below 150mW/cm² delivered by either an argon ion or copper vapour pumped dye laser. A microlens attachment to the optical delivery fibre ensured even light distribution. A multi-jointed articulated arm capable of easy manipulation in three planes was used to hold the fibre to facilitate light delivery. The distance from the surface of the lesion at which the fibre is positioned is apt to change with any movement of the patient's head, tongue or lower jaw. This position was therefore constantly checked with a depth probe to ensure accurate light dosimetry.

The first three patients were admitted overnight but with increased understanding of the technique, subsequent patients were treated as out-patients. No anaesthetic was required during the treatment, but analgesia was frequently required during healing. All lesions were photographed before and at

a series of times after treatment to document macroscopic tissue response, patients being seen every two to three days following PDT until the lesions were excised. Following sensitisation, all patients were counselled on the need to manage re-exposure to light.

Between two and 19 days following PDT, the treated lesions were excised, fixed in 10% buffered formalin and processed with paraffin. Sections were stained with haematoxylin and eosin (H&E) and by the elastin-Van Gieson (EVG) method and a reticulin stain. Selected sections were further stained for cytokeratin using a routine immunoperoxidase method and antibodies to human callus keratin (Dako, High Wycombe, UK) and cytokeratin (MN116; Dako).

The depth of the PDT effect was measured on low-power colour photomicrographs using a computer-aided image analysis system (Quantimet). Absolute measurements were determined on each photograph by direct reference to the original H&E-stained section. Two distinct depths were measured at several points to determine a mean value:

Depth of absolute necrosis: this was the thickness of the surface necrotic slough, defined as the distance from the surface to the base of the surface necrosis, within which no viable cells, either normal or tumour, could be distinguished.

Depth of inflammation: this was defined as the depth of tissue showing evidence of an inflammatory response measured from the surface of the necrosis to the point where there was least evidence of inflammation. Inflammation was defined as the presence of extravascular acute or chronic inflammatory cells and the presence of polymorphonuclear leukocytes within dilated blood vessels.

7.2.2 Results

7.2.2.1 Clinical

Clinical examination of the treated areas showed no macroscopic difference following PDT in the appearance of tumour or its surrounding normal mucosa, both being replaced by an ulcer slough within 24 hours. The dimensions of this area of slough closely corresponded to the area exposed to laser light. This is illustrated in Figure 7.1 (a case not included in the excision following PDT protocol as no follow up excision or biopsy was performed) which shows PDT response and healing of lip malignancy and surrounding normal tissue.

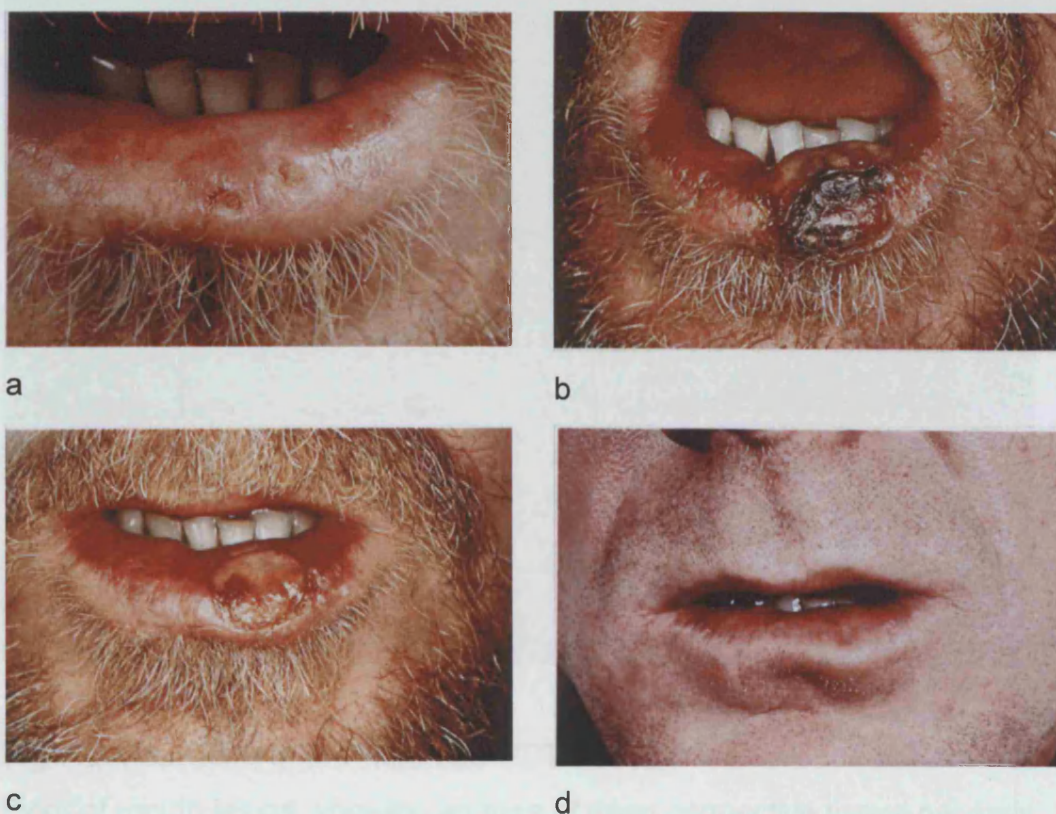


Fig 7.1: Two small carcinomas of the lip (a) before treatment and (b) four days after PDT (Photofrin® 2mg/kg followed by 50J/cm² of red light at 630nm, 48 hours later). There is necrosis covering the entire area exposed to light, which included both normal and malignant tissue. (c) Eighteen days after PDT, healing is well advanced. (d) Thirty-three days after PDT, healing is complete. There is a small scar in the PDT-treated area, where a biopsy had been taken prior to treatment; but apart from this, healing has been by regeneration of normal tissue with an excellent cosmetic result. In contrast, in the region on the

opposite side where a small carcinoma was previously removed by wedge excision, there is distortion of the lip contour.

7.2.2.2 Histopathology

Both tumour and adjacent epithelium were replaced by an amorphous eosinophilic fibrinopurulent slough, confirming the macroscopic findings. Throughout the mucosa there was connective tissue necrosis with marked eosinophilic fibrinous exudates and varying degrees of inflammatory cell infiltration (neutrophils, lymphocytes and eosinophils, Figure 7.2).

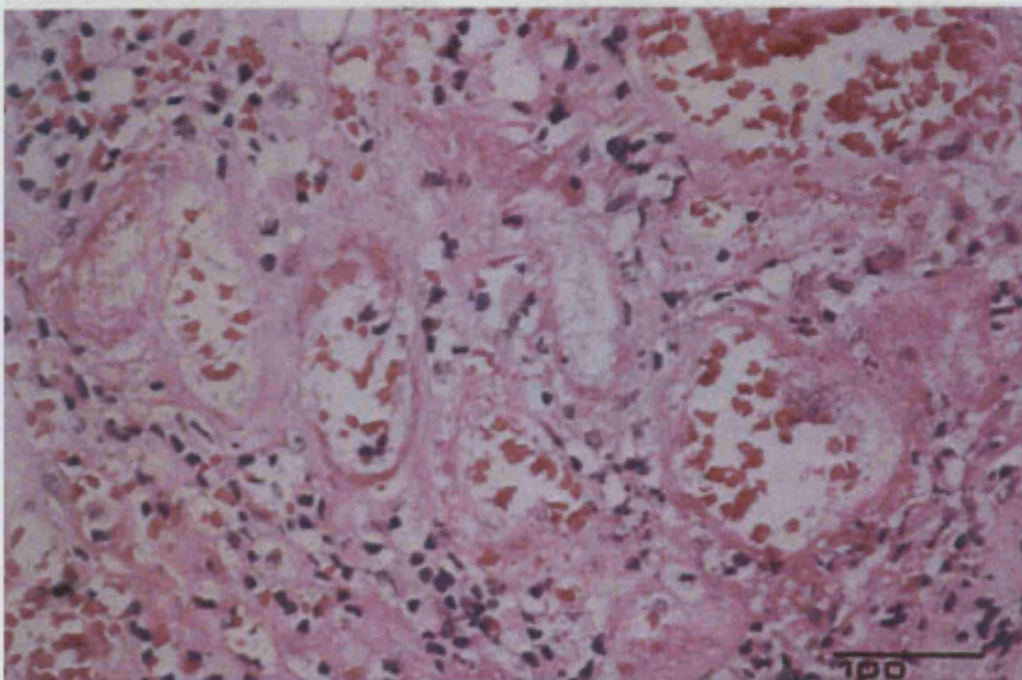


Fig 7.2: Four days after Photofrin® PDT

Floor of mouth lesion, showing an area of deep connective tissue necrosis. There are extravascular fibrinous exudates associated with infiltrates of inflammatory cells. The blood vessels show loss of an endothelial lining and replacement of the wall with a hyalinised eosinophilic coagulum. Most vessels remain patent. H&E (bar = 100µm).

Loss of endothelium in the small blood vessels was common with signs of haemorrhage, thrombosis and fibrinous exudate, although most vessels appeared patent in spite of obvious injury. Larger vessels showed replacement of the wall with a hyalinised eosinophilic material with loss of cellularity. No

evidence of selective tumour necrosis was found. In routinely stained sections, necrotic tumour could not be distinguished from the adjacent necrotic connective tissue. However, with the aid of immunohistochemical cytokeratin staining techniques, necrotic tumour could be distinguished from the connective tissue necrosis observed, and reticulin staining helped to distinguish the outline of the original epithelium and its basement membrane (Figure 7.3).

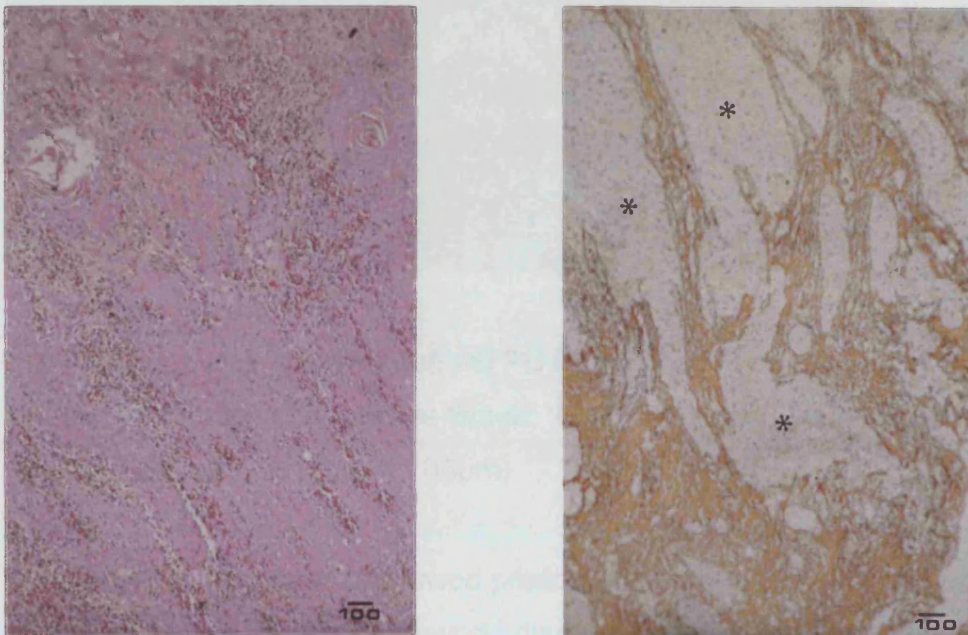


Fig 7.3: Two days after Photofrin® PDT on a floor of mouth lesion
Serial sections of necrotic tumour stained with (left) H&E and (right) a reticulin stain. The superficial tumour is completely necrotic but is well delineated as pale areas (*) surrounded by reticulin-positive connective tissues (bar = 100µm)

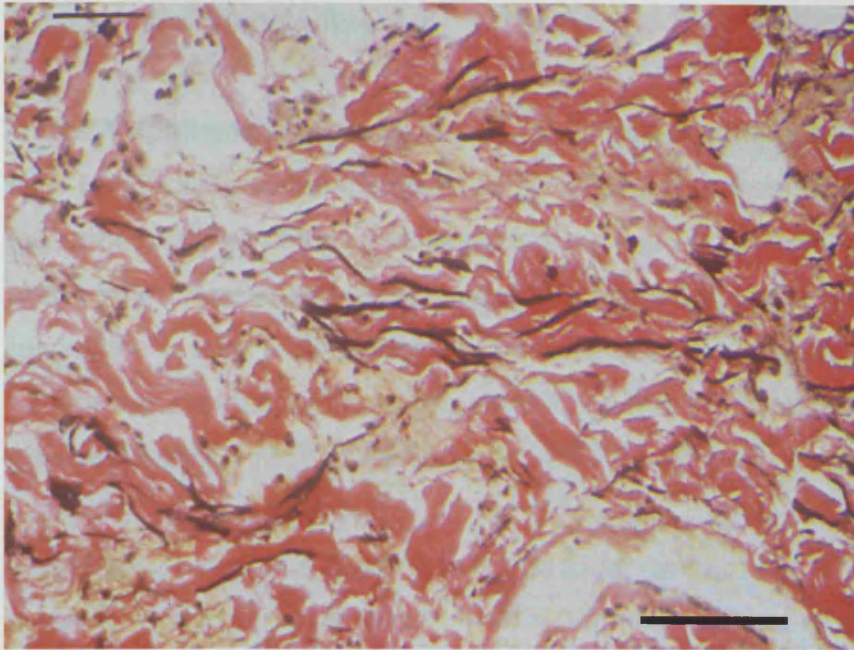


Fig 7.4: Four days after Photofrin® PDT on a floor of mouth lesion
An area of inflamed connective tissue. Mature collagen (red) and elastic fibres (black) are preserved (bar = 100µm)

In all cases, EVG staining showed preservation of mature collagen and the elastin structural protein framework, disrupted only occasionally by oedema and inflammation (Figure 7.4).

In four cases, viable tumour was seen within the resected specimen. In one this was seen at the resection margin not exposed to light, but in two, viable tumour was encountered beneath the area of necrosis. In one case, resected after two days, residual viable tumour islands were noted within an area of necrotic connective tissue and fibrinous slough. It was felt that this would eventually have undergone sloughing with time had it not been excised.

Beneath the epithelium, connective tissues showed gradually decreasing necrosis, oedema and inflammatory cell infiltration. In all cases, necrosis and heavy inflammation extended through the full thickness of the mucosa.

7.2.2.3 Quantitative Results

Of the 11 patients treated, seven had satisfactory complete PDT ablation of their tumours in the resected specimen with tumour-free surgical margins. Of the remaining four, three had viable tumour islands present at depths of 4.0, 2.5 and 1.3mm. These were resected at 2, 5 and 5 days post-treatment respectively, after PDT necrosis but before sloughing was complete. The fourth patient had residual tumour at one resection margin, for which he subsequently received curative radiotherapy.

The absolute necrosis depth was found to range from 1.1 to 4.1mm below the surface, with a mean value of 2.1 (sd = 0.9)mm. In most cases, this depth was found to be the same as the depth at which evidence of microvascular injury could be seen. The depth of inflammation was considerably deeper, ranging from 2.8 to 9.9mm, with a mean of 4.8 (sd = 1.8)mm (see Table 7.1).

Patient	Site	Excision (days post-PDT)	Depth absolute necrosis, mm (\pm sd)	Depth inflammation, mm (\pm sd)	Presence of slough	Depth of viable tumour below surface, mm
1	FoM	2	4.1(\pm 0.3)	9.9(\pm 0.1)	No	4
2	FoM	5	1.5(\pm 0.1)	4.3(\pm 0.1)	Yes	1.3
3	FoM	3	2.3(\pm 0.1)	5.1(\pm 0.2)	No	-
4	T	5	2.2(\pm 0.7)	5.8(\pm 0.7)	No	2.5
5	FoM	7	1.1(\pm 0.1)	3.3(\pm 0.0)	No	Margin
6	FoM	2	2.9(\pm 0.1)	5.6(\pm 0.5)	No	-
7	FoM	5	2.6(\pm 0.1)	5.0(\pm 0.0)	No	-
8	BM	19	1.3(\pm 0.1)	2.9(\pm 0.1)	Yes	-
9	FoM	6	1.7(\pm 0.2)	3.4(\pm 0.4)	Yes	-
10	A	5	1.9(\pm 0.1)	2.8(\pm 0.1)	Yes	-
11	T	6	1.1(\pm 0.1)	4.8(\pm 0.4)	Yes	-
			Mean 2.1(\pm 0.9)	Mean 4.8(\pm 1.7)		

Table 7.1: Details of tumour location, time of excision and quantitative histological findings

Key: FOM – Floor of mouth, A – Alveolus, T – Tongue, BM – Buccal mucosa

7.2.3 Discussion

This study did not support the widely held view that PDT using dihaematoporphyrin ether/ester (Photofrin®) causes selective tumour destruction without normal tissue injury. In the literature the range of light doses used in the treatment of oral mucosal lesions varies from 50J/cm² to 1,620J/cm² (Zhao et al 1991, Wile et al 1983, Carruth 1990, Calzavera et al 1989, Feyh 1992, MacCaughan 1987, Wenig et al 1990). At higher levels the power densities used may also cause thermal injury to tissue, which may well be clinically beneficial but which makes it difficult to assess the contribution of the photodynamic effect.

Light dose regimes have not yet been standardised and light doses used in various studies are arbitrary. However, a light dose of 50J/cm² represents the lower limit that has been shown to result in tumouricidal effects in PDT of upper aerodigestive carcinomas, and consequently this dose was chosen for this study.

This dose also should allow the detection of any evidence for selectivity of response and should spare normal tissues from injury. Our comparison of pre- and post-PDT photographs of treated lesions showed no evidence of a macroscopic difference in response of tumour or its surrounding normal mucosa; both were replaced by an ulcer slough, making the tumour site indistinguishable from the adjacent normal tissue. The dimensions of this slough closely corresponded to the area exposed to laser light. It would appear, therefore, that the tumour does not respond any differently from the surface epithelium. Monnier et al (1990) noted superficial ulceration of normal buccal mucosa using light doses of 60J/cm² 72 hours following HPD administration. However, these were macroscopic observations alone. There was no histological evidence of selective tumour necrosis. Indeed, it was only with the aid of special staining techniques that necrotic tumour could be distinguished from the adjacent necrotic connective tissue.

As explained in 4.1.4, photochemical injury results in a different type of injury from that observed with heat injury created by thermal lasers or diathermy. This

non-thermal injury does not damage or denature the sub-epithelial collagen and elastin, and reticulin staining confirmed that there was preservation of non-cellular supporting elements, suggesting that the tissue architecture may be maintained while cellular and vascular elements are damaged. This may provide a matrix for regeneration of normal tissues and may explain the excellent healing observed both in clinical and animal studies. Similar observations have been made in the colon, bladder and trachea in rats and in mucosal surfaces and skin in patients (Barr et al 1987a, Pope et al 1991b, Smith et al 1993). This might be interpreted as selective tumour necrosis, whereas in fact there is necrosis of both tumour and normal tissue, with ultimate healing by regeneration of normal tissue.

All resected specimens showed evidence of microvascular injury with loss of endothelium and a 'fibrinoid' type of necrosis in larger vessel walls. These changes were accompanied by the presence of fibrin in the necrotic zones, suggesting an increase in vascular permeability. Only a few of the vessels in the lamina propria and sub-mucosa were actually thrombosed, and many appeared patent. This confirms the view published elsewhere that microvascular injury is not solely responsible for tissue necrosis and that direct cytotoxic injury may also occur.

The presence of islands of viable tumour at relatively superficial levels is a cause for concern. But, in three cases, the tumour was surrounded by extensive connective tissue damage and vascular injury with thrombosis, and it is probable that sloughing or eventual ischaemic necrosis would eventually occur.

Viable tumour was also found at a deeper level than the necrosis, and this would not be affected, due to the attenuation of penetrating light (Bown 1990, Gluckman 1991b). In these cases, tumour survival is inevitable, and rapid epithelial regeneration following PDT could mask the need for further intervention. Pre-treatment tumour imaging is clearly extremely important and if PDT is to be used for such deep lesions, then light delivery via interstitial fibres may be necessary.

PDT-induced inflammatory changes extended to depths from 2.8mm to 9.9mm at this light dose. This variation probably reflects variations in the optical properties of the tissues or tumours being treated, and difficulties in delivering light uniformly to the irregular surfaces in the mouth.

There was always necrosis to the full thickness of the oral mucosa. Therefore, even at this light dose one can be confident of destroying lesions that are confined within this level, such as severe dysplasias, carcinoma *in situ*, and early invasive lesions which have not penetrated to the muscle or periosteum.

All patients tolerated PDT without analgesia or significant discomfort at the time of treatment. After the first three patients were treated as in-patients, the remainder were treated on a day-care basis. Good compliance with instructions regarding avoidance of direct sunlight, resulted in no patient experiencing any significant cutaneous phototoxic event, though minor skin erythema and irritation of the hands and face was experienced by three patients. Most of the patients experienced some degree of tanning, which persisted for several months.

Pain was experienced at some stage following PDT by all of the patients and in some instances necessitated oral opiate analgesia. The use of a local analgesic and anti-inflammatory spray prior to meals was also found to be useful. In most cases pain commenced 24 to 48 hours after completion of the treatment and lasted for several days.

7.2.4 Conclusion

True selective tumour necrosis could not be observed using Photofrin® in this study. The depth of necrosis ranged from 1.1 to 4mm below the surface, which may be inadequate to clear all tumour cells in superficial cancers of the oral cavity. Residual photosensitivity was long but in this study led to no serious adverse effects.

7.3 PDT Effect on Human Tissue with ALA

ALA is now licensed in the USA for use in its topical form in the treatment of early basal cell carcinomas and actinic keratosis. In this preparation there is no cutaneous photosensitivity except at the local site where the drug is applied and even when administered systemically there is only a short period (1 to 2 days) of cutaneous photosensitivity (Mustajoki et al 1992). Healing following treatment is excellent making it therefore ideal to treat extensive dysplastic lesions in the oral cavity. In this series of studies, systemic administration of ALA was given as we were not able to find an effective topical preparation for intraoral use.

7.3.1 Biodistribution Study

Pre-clinical studies were undertaken initially to elucidate the biodistribution of protoporphyrin IX the photoactive derivative of ALA. In these it was clearly shown that protoporphyrin IX accumulated preferentially in the epithelium with little uptake in the underlying muscle. This raised the possibility of selective epithelial damage inflicted by PDT with no damage to the deeper tissue.

Local ethical committee approval was granted for this study, a Medicines Control Agency "Doctors and Dentists Exemption from Clinical Trials Certificate" was granted, and informed consent was obtained in each case. 4 patients with advanced squamous cell carcinomas of the mouth were given 30-60mg/kg ALA by mouth then kept in a semi-darkened room for 24h. Serial biopsies were performed before and up to 24h after ALA. 10µm frozen sections were looked at by quantitative fluorescence microscopy (corrected for autofluorescence) as described by Grant et al 1993a. Maximum fluorescence was found 4-6h after ALA, and had returned to background levels in 24h (figure 7.5). Tumour fluorescence intensity in epithelium was about twice that of the surrounding connective tissues, showing the selective ability of tumour tissue to synthesise and accumulate porphyrins. This differs from the accumulation of phthalocyanines which is concentrated in the submucosal connective tissue (figure 7.6). Where normal or dysplastic epithelium could be sampled, this was seen to behave similarly to tumour areas. High performance liquid

chromatography done in one case confirmed the fluorescing porphyrin to be >96% PPIX, representing 2-2.5µg/g of tissue at peak fluorescence.

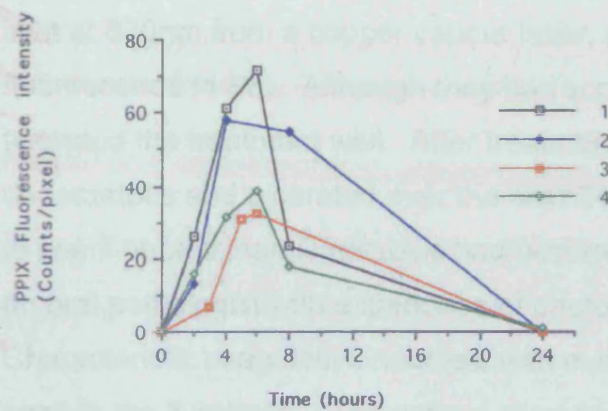
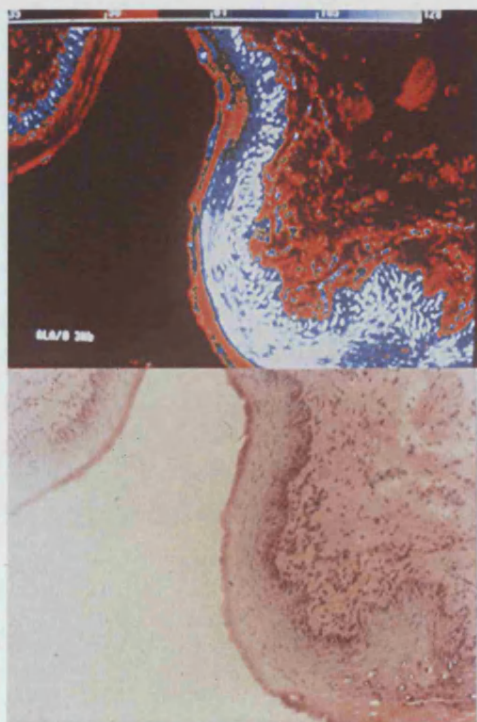


Fig 7.5: Tumour protoporphyrin IX fluorescence with time in 4 patients



ALA

Fig 7.6: Fluorescence images for ALA with histological comparison

Note that ALA fluorescence is maximal in the epithelium as shown by the cold false colour image.

7.3.2 Histological Effects

Three patients (the 4th was responding to radiotherapy) were given 60mg/kg of ALA on another occasion and their tumours were exposed to 50-100J/cm² laser light at 630nm from a copper vapour laser, at times corresponding to maximum fluorescence (4-6h). Although they had some local discomfort, patients tolerated the treatment well. After treatment, the surface of the tumour became oedematous and ulcerated over the next 24h. Biopsies were performed at 48h to see if photodynamic necrosis had occurred. Specimens were examined by an oral pathologist with experience of photodynamically-induced injury. Characteristic coagulative necrosis with evidence of microvascular injury was seen in the 3 patients. The pattern of necrosis was entirely as predicted from the fluorescence studies – that is to say necrosis limited to the epithelium where the PPIX was concentrated. There also seemed to be little vascular damage (figure 7.7) Treatment was palliative and complete tumour ablation was not an aim in these advanced cancers. Treatment with ALA did not cause abnormalities in electrolyte balance nor haematological indices. 3 of the 4 patients showed a rise of serum aspartate aminotransferase concentrations, which returned to normal within 3 days. 2 had a small temporary rise in total bilirubin.

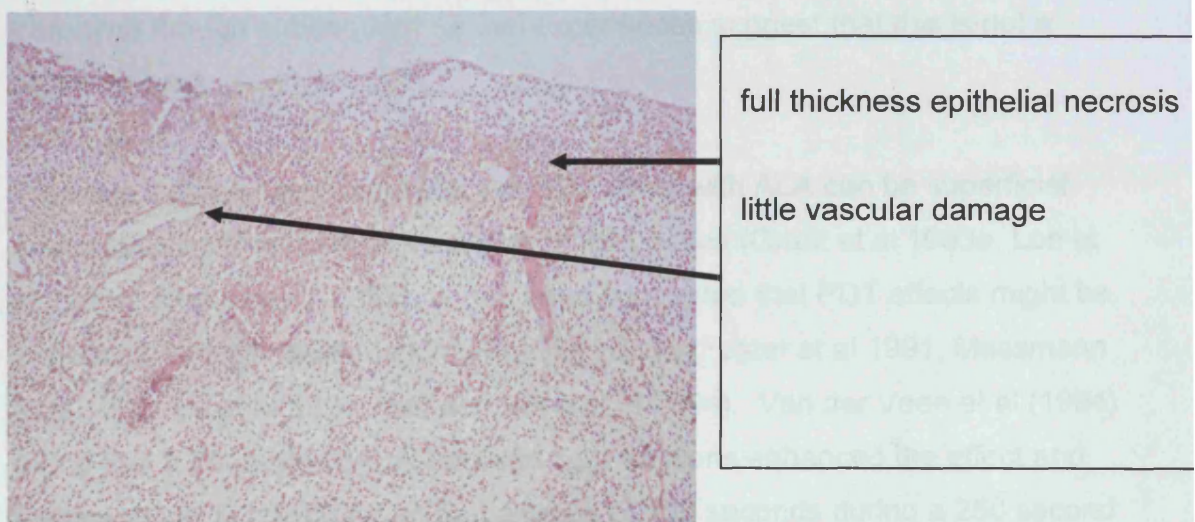


Fig 7.7 Histological effects of ALA PDT

This study shows that oral squamous cell carcinomas can synthesise and accumulate photosensitising levels of PPIX after oral ALA. Only tumour

samples were removed from the mouth to measure fluorescence, and normal tissue sensitisation was not examined, although some necrosis of normal mucosa next to the tumour and exposed to light was seen. The main advantage of using ALA-induced PPIX for photosensitisation is the rapid clearance of the sensitiser within 24h, which would make it possible to repeat treatments at short intervals until ablation is complete. Giving ALA by mouth is more acceptable to the patient than by intravenous injection. The use of systemic ALA to induce PPIX photosensitisation is a novel method of tumour photodynamic therapy, which may yet prove suitable as a primary treatment for early cancers and dysplasias of the oral cavity.

7.4 Further Studies to Determine Depth of Effect Following ALA PDT

A further group of patients was treated using systemic administration of ALA

7.4.1 Materials and Methods

The study was conducted on 15 patients using oral preparations of ALA. All patients had histological confirmed malignant or pre-malignant lesions in the mouth but none had regional nodal disease. Patients with active liver disease were excluded from the study because of the risk of precipitating acute porphyria though subsequent clinical experiences suggest that this is not a significant risk.

Previous studies have found that the PDT effect with ALA can be superficial even with long treatment times and high light doses (Grant et al 1993a, Loh et al 1993a, Regula et al 1995). It has been suggested that PDT effects might be enhanced through fractionation of the light dose (Foster et al 1991, Messmann et al 1995, Pe et al 1994, Van den Veen et al 1994). Van der Veen et al (1994) found that a 75 minute break between light fractions enhanced the effect and Messmann et al (1995) found that a break of 150 seconds during a 250 second exposure enhanced the PDT effect substantially compared to the same light dose delivered without a break.

Details of treatments are given in table 7.2. The study had approval from the UCL Ethics Committee and treatment was carried out with informed consent. Initially patients were treated as inpatients but subsequent treatments were carried out on an out-patient basis. The photosensitiser used in this project was supplied by DUSA Pharmaceuticals Inc (Wilmington, MA). All preparations were made up in orange juice and given in three divided doses of 20mg/Kg at 0, 1 and 2 hours following the regimen established by Regula et al (1995). All patients were kept away from bright sunlight for 24 hours following drug administration and a drug light interval of 2.5 hours was used (4.5 hours after the first dose of ALA).

Laser and Light Delivery: A variety of light sources were used. The gold vapour laser giving light at 628nm (Dynamic Light Ltd, Milton Keynes, UK) was used. This was delivered using a mode scrambled 400um core flexible optical fibre with a bare plain cleaved tip giving a circular spot up to 2.5cm in diameter. Power dosing was kept below 150mWatts per cm² to avoid thermal effects although this meant that treatment times were long (up to 143 minutes) so maintenance of patient and fibre position was sometimes difficult. The irradiation regime was varied a little with the first group consisting of eleven patients who received a total light fluence of 100J/cm² in two fractions with a minimum of 5 minutes between the two fractions (after Messmann et al 1995) and a second group of four patients who received 200J/cm² in two fractions delivered at 2.5 and 4 hours after the first dose of ALA (after van der Veen et al 1994).

7.4.2 Results

Patient	Age/Sex	Site	Previous Treatment	Maximum Necrosis/mm	Maximum Inflammation mm/layer
Group 1					
1	73/M	BM	Nil	0.9	1.2/submucosa
2	69/M	FM	CO ₂	0.8	1.5/submucosa
3	52/M	FM	Nil	1.1	6 ⁺ /muscle
4	79/M	T	Nil	0.6	2.5 ⁺ /submucosa
5	51/M	FM	Nil	0.1	1.0/submucosa
6	75/M	BM	CO ₂ laser	0.6	1.5/mucosa
7	70/M	BM/A	Nil	0.3	1.2/submucosa
8	87/F	T	Field change	0.5	1.2/muscle
9	81/F	A	mandibular resection	0.5	1.0/mucosa
10	62/M	FM	Nil	1.3	Not measured
11	65/M	BM/A P/RM	Field change	0.7	1.3/submucosa
Mean				0.7mm	1.8mm
Group 2					
1	67/M	BM	Nil	0.5	1.1/submucosa
2	46/M	A	CO ₂ laser	0.2	0.9/submucosa
3	49/M	T	CO ₂ laser	0.3	0.8/muscle
4	59/F	T	CO ₂ laser	0.6	2.5/muscle
Mean				0.4mm	1.3mm

Table 7.2: Details of tumour location and quantitative histological findings

Key: Pt-patient; A-alveolus; BM-buccal mucosa; FM-floor of mouth; P-palate; RM-retromolar trigone; T-tongue;

+indicates inflammatory changes extending through full thickness of biopsy;

Group 1 received 100J/cm² in two fractions with 5 minutes between the two fractions

Group 2 received 200J/cm² in two fractions delivered at 2.5 and 4 hours after the first dose of ALA

Biopsies were taken between 3 and 8 days post treatment



Fig 7.8: 4 days after illumination

The treated area became inflamed within a few hours. This was followed by the formation of a whitish slough of the superficial layers after 1-2 days. Healing then took place over the next three to five weeks (larger lesions taking longer to heal than smaller ones) with no evidence of scarring except at biopsy sites.

Depth of PDT Damage: In the first group of eleven patients – the group that received a total dose of $100\text{J}/\text{cm}^2$ with a five minute break in light delivery at the mid point produced a depth of necrosis varying from 0.1 to 1.3mm (mean 0.7mm). The depth of inflammatory response ranged from 1 to 6mm (mean 1.8mm). Again full thickness epithelial necrosis was seen in all cases with a variable depth of inflammatory response.

In four patients in group 2, ie; those that received a total light dose of $200\text{J}/\text{cm}^2$ with a 90 minute break between light fractions, the depth of necrosis was 0.2 to 0.6mm (mean 0.4mm). Inflammatory changes extended from 0.8 to 2.5mm (mean 1.3mm). Epithelial necrosis was seen in all cases.

The depth of PDT effect with ALA seems to be limited to the epithelium. This is entirely as predicted from the fluorescence studies which show that there is preferential accumulation of protoporphyrin IX (PPIX), in the epithelium only with relative sparing of deeper tissues. This certainly implies that it might be

difficult to get a very deep PDT effect using ALA for cancer but it might be effective in the treatment of pre-malignancy.

7.4.3 Discussion

PDT using ALA has a shorter period of cutaneous photosensitivity than with Photofrin®, making it more acceptable to clinicians and patients.

The depth of necrosis with ALA was found in this study to be between 0.1 and 1.3mm and the inflammation ranged between 0.8 and 6mm. Both of these are shallower than the findings with Photofrin® (Section 7.2).

Although it was difficult to measure the depth of necrosis accurately due to variations in the time between PDT and biopsy (3 – 8 days), it was clear that there was complete epithelial necrosis in every case and no necrosis was seen in muscle - although some cases showed inflammation in muscle.

There was no selectivity of necrosis between normal and abnormal epithelium - the area of necrosis always corresponded to the illuminated area. There was however selectivity of necrosis between epithelium and underlying muscle - consistent with the fluorescence microscopy findings which showed that there were much lower levels of PPIX in underlying connective tissue and muscle.

The maximum dose that patients can tolerate orally is 60mg/kg (Regula et al 1995) which, by extrapolation from animal studies (Messmann et al 1995) may be only just above the threshold dose required to produce a PDT effect (50mg/kg). An iv formulation of ALA might halve the dose required to achieve target tissue levels (Loh et al 1993) and the maximum tolerable dose may be higher leading to increased tissue levels of PPIX and a greater depth of necrosis. This does not seem to be the case however in the small number of post IV ALA PDT biopsies undertaken to date.

The maximum depth of necrosis in this study was 1.3mm which clearly limits the use of ALA PDT to superficial carcinomas and dysplasia. It would be preferable if ALA could produce a greater depth of necrosis as its short cutaneous

photosensitivity is an attractive feature. It might be possible to further increase the IV drug dose to optimise treatment effects. This is clearly a possible way forward.

7.5 PDT Effect with mTHPC (Foscan®)

7.5.1 Introduction

Photodynamic therapy using Photofrin® has been shown to be of benefit but it is limited by a weak absorption band in the red region of the spectrum and a long period of residual photosensitivity. ALA has a shorter period of residual photosensitivity (two days) but this is offset by a smaller depth of necrosis.

Meta-tetrahydroxyphenyl chlorin (mTHPC, Foscan®: (***Biolitec***) Scotia Pharmaceuticals, (***Ireland***) Dublin) appears to have a number of advantages over both of these photosensitisers.

The aim of this study was to determine the histological changes of mTHPC photodynamic therapy, in a group of patients with oral squamous cell carcinomas or severe epithelial dysplasia.

7.5.2 Material and Methods

Observations were based on a group of 10 patients treated and described in Chapters 8, 9 and 10. That is, patients with varying degrees of dysplasia, early invasive cancer and field change disease.

Treatment protocol involved the intravenous infusion of 0.15mg/Kg of mTHPC which was administered through a filter 96 hours prior to laser irradiation. Illumination was carried out using a copper vapour pumped dye laser emitting light at 652nm (Dynamic Light, Hornsby, Australia) with a beam delivered via a 0.5mm diameter flexible microlens system (PDT Systems, Santa Barbara, California) to obtain the light dosimetry. Later a diode laser (Diomed, Cambridge, UK) was used to effect treatment. The irradiance was kept below 250mWatts per cm² to avoid thermal effects and biopsies were taken wherever possible at four separate times, if the patient was able to tolerate it. That is to

say at initial diagnosis, immediately prior to irradiation, during the ulcerated stage and when healing is complete. The application of local anaesthetic was limited to the site of biopsy to avoid any possible alteration in the PDT effect. Specimens were fixed in 10% neutral buffered formalin and processed routinely then 5µm sections were cut and stained with haematoxylin and eosin for histopathological examination. Routine haematological and biochemical investigations were carried out before and after mTHPC administration.

7.5.3 Photodynamic Therapy

Treatments were carried out with the patient conscious in all except one case (patient 14) where a general anaesthetic was administered to improve access to a lesion on the soft palate. The treatment times were short, ranging from 113 to 480 seconds, and treatment was generally well tolerated. The lesion plus a 1 to 2cm margin of normal tissue was treated. This area was marked with small dots of crystal violet blue dye to allow repositioning or resizing of the irradiated spot, should patient movement occur.

Patients were kept in a dimmed room after receiving mTHPC, and given verbal and written instructions on avoidance of bright light during the two to three weeks for which they were likely to be photosensitive.

7.5.4 Results

7.5.4.1 Depth of PDT Effect

Although we were able to make an assessment of the depth of PDT effect with ALA and Photofrin®, this proved very much harder with Foscan®. Post treatment biopsies were taken, but the tissue was often so severely affected that meaningful interpretation was not possible, however, the overall depth of PDT effect was much deeper than with Photofrin® and of the order of 1cm which is in keeping with other estimates. This will in part be a function of greater light penetration at 652nm (Foscan®) compared with 635nm (Photofrin®) and also the greater quantum yield of free radicals generated by Foscan®.

7.5.4.2 Clinical Course

Twenty-eight lesions were treated in 26 PDT treatment sessions. Within a few hours of PDT, the treated area exhibited erythema, oedema and in some cases blister formation. This was followed by a phase characterised by vascular stasis and congestion, leading to necrosis, the formation of a fibrino-purulent slough and ultimately ulceration. The ulceration typically took five to eight weeks to heal, with some scarring in five patients. These effects generally improved with time (Fig 7.9: 1a to d), although two patients developed complications. One of these had a very large T₄ tumour (32cm²) on the palate irradiated with 20J/cm². Reflected and scattered light resulted in ulceration and necrosis of normal oral tissue well away from the target lesion. Normal tissues in subsequent cases were shielded to avoid this.

The second problem was significant tethering of the tongue after treatment of a carcinoma *in situ* in the floor of the mouth. As can be seen by the following case this situation improved over time.



Fig 7.9: (a) T₁ squamous cell carcinoma and surrounding leukoplakia of the ventral tongue/floor of mouth before photodynamic therapy (b) 13 days after

Foscan® photodynamic therapy showing ulceration with the presence of a thick fibrino-purulent slough of the entire irradiated site which included normal and abnormal regions (c) Seven weeks after PDT with complete healing of the treatment site. Scarring is present (hyperplasia was seen on the post-treatment biopsy) (d) Eight months after PDT showing disappearance of the scarring at the treatment site

Analgesia was required by all patients in the early ulcerative phase, starting one to two days post-PDT, with oral opiates being prescribed for one to two weeks for most patients. All patients had a full blood count, serum electrolytes and liver function tests, before and up to three months after administration of mTHPC. There were no changes in either the haematological or biochemical indices following PDT.

7.5.4.3 Pathological Changes

Biopsies taken in the early stages (three to seven days), show lesions characterised by massive acute inflammation, vascular damage and oedema. There was ulceration of the overlying epithelium and necrosis, which frequently extended to the submucosa and occasionally into muscle. There was prominent separation of muscle fibres by inflammatory cells and oedema fluid with scattered infiltrates of eosinophils (Figure 7.10).

The mucosa was replaced by a dense oedematous fibrino-purulent slough, with a massive exocytosis of neutrophils and occasional eosinophils (Figure 7.11). Small blood vessels, arterioles and venules showed loss of endothelial cells and prominent pavementation of polymorphonuclear leukocytes (Figure 7.12). The acute inflammatory infiltrate and oedema extended deep into the underlying submucosa and muscle when present.

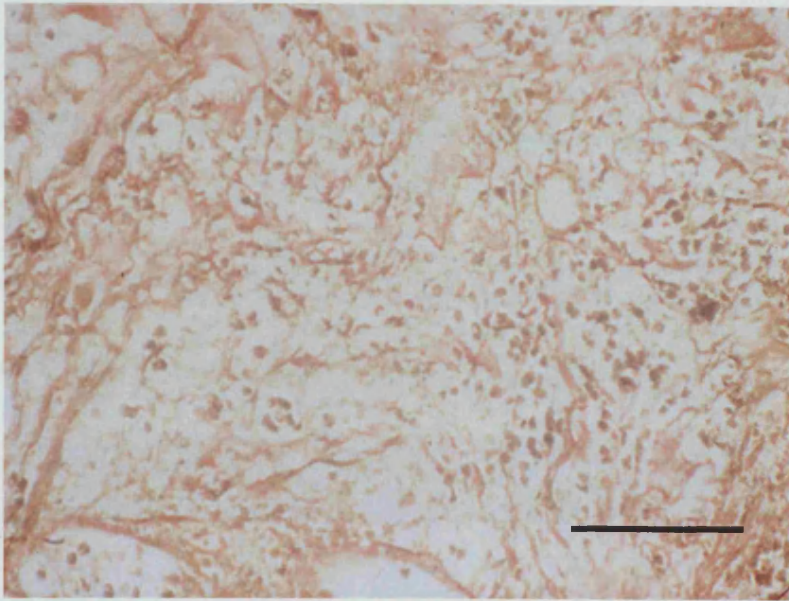


Fig 7.10: Three days after Foscan® PDT

Disruption of normal tissue architecture – note disordered collagen, elastin and muscle with infiltration of inflammatory cells (bar = 50µm).

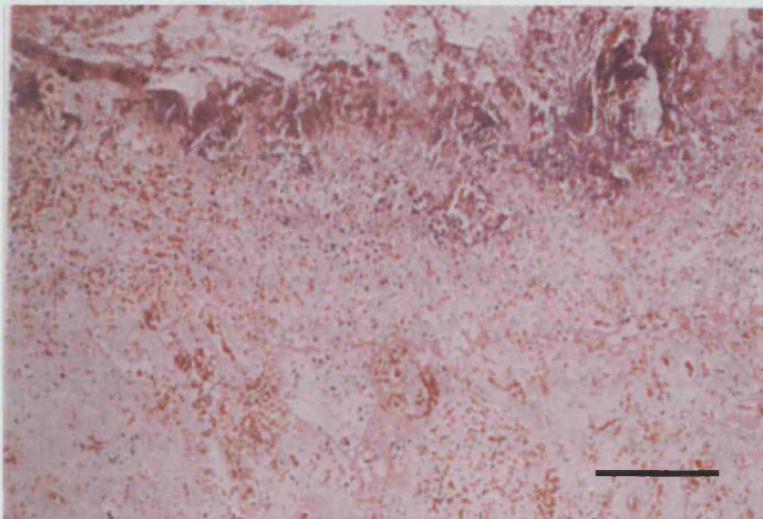


Fig 7.11: Characteristic findings three days after PDT

The epithelium is completely replaced by necrotic ulcer slough and the connective tissue is oedematous and shows inflammation and haemorrhage. H&E. (bar = 50µm)

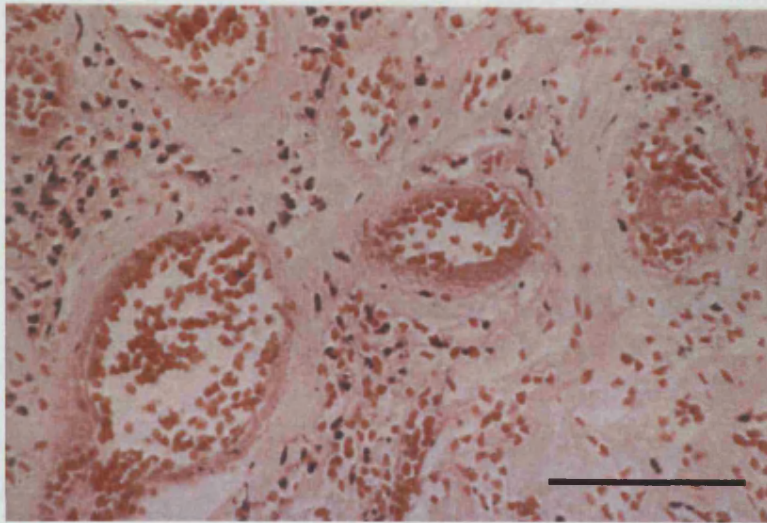


Fig 7.12: Characteristic changes in blood vessels three days after PDT

There is extensive vascular damage with loss of endothelial cells and resulting oedema, extravasation and inflammation. Thrombus formation is prominent. H&E. (bar = 50µm)

In biopsies taken at 10 to 12 days there was still evidence of widespread ulceration and mucosal necrosis with acute inflammatory cells, but infiltrates of lymphocytes and macrophages were few at this stage. At the margins of the ulcerated area, growth of new epithelium was frequently seen below the fibrino-purulent slough. Deep below the mucosa, and often within muscle, there was deposition of immature granulation tissue with proliferating endothelial cells, fibroblasts and chronic inflammatory cells. This process of organisation suggested that the end result would be scarring, which was consistent with the clinical findings and was seen in specimens taken after healing was complete (Figure 7.13).

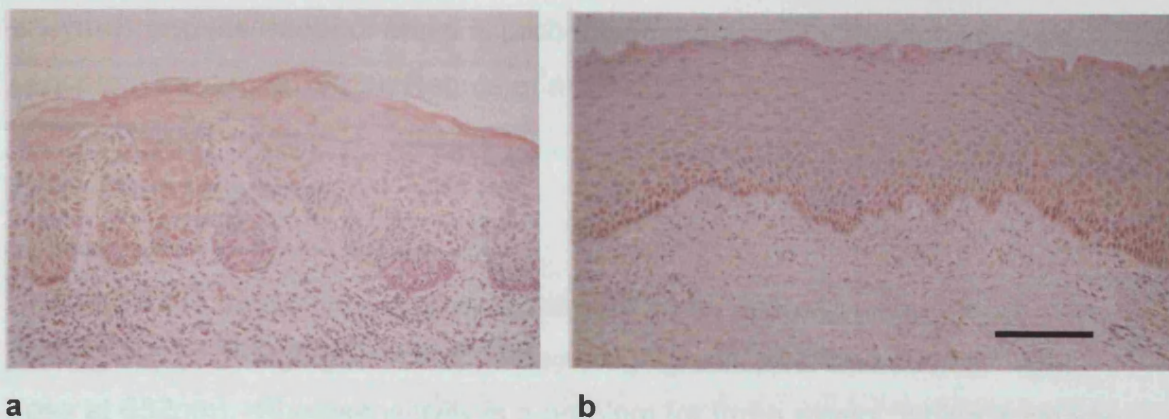


Fig 7.13: (a) Biopsy showing severe epithelial dysplasia with evidence of microinvasion before PDT. H&E; bar = 50µm. (b) Biopsy of the same site three months after PDT showing healing with well-ordered epithelium overlying fibrous scar tissue. H&E; (bar = 50µm)

7.5.5 Discussion

PDT using mTHPC has a very powerful effect which appears to be directly cytotoxic with some elements of vascular stasis. The disruption to the collagen and elastin would suggest that post-treatment scarring is likely and has in fact been shown to be the case although over time these changes subside to more acceptable levels. (Figure 7.9: a to d).

It is vital that the depth of PDT necrosis be greater than the depth of each individual tumour. Surface irradiation of deep tumours may leave residual tumour beneath regenerated mucosa, as the red light used to activate the photochemical reaction only penetrates a few millimeters. The depth of light penetration, and hence sensitiser activation is important whether the mechanism of cell kill is direct cytotoxicity or destruction of the tumour vasculature. From the histopathology, vascular damage plays an important role in the patients described in this study (Figure 7.12).

Although PDT with mTHPC was successful in the treatment of the oral dysplasias, ALA also adequately eradicates this superficial disease as shown in the previous section (7.3)). ALA has the advantage of inducing only one to two days of cutaneous photosensitivity, but it may cause elevated levels of liver

enzymes and the depth of effect is probably inadequate for treating invasive cancers (Grant et al 1993a, Regula et al 1995).

7.5.6 Conclusion

Foscan® PDT causes effective necrosis with some scarring using these parameters (0.15mg/kg Foscan®, 4 day drug light interval and 20J/cm² light dose at 652nm). Photosensitivity is a problem for three weeks, which is clearly inferior to ALA but much better than Photofrin®.

7.6 Comparison of the Three Sensitisers

There were a number of problems common to all three drugs when used for PDT treatment in the oral cavity. Accurate light dosimetry within the mouth is extremely difficult due to the undulating contours of the mouth and the likelihood of movement, especially of the lower jaw and tongue.

More posteriorly it is difficult to ensure that light is delivered at right angles to the surface. 1A divergent microlens allows even light emission from the fibre and allows the fibre to be placed closer to the surface but in this region of the mouth, occasionally elliptical light spots must result. As the power density of the light varies inversely with the square of the distance between the fibre and the target tissue, even small variations in the distance from the fibre tip from that originally calculated will result in large differences in the total energy density delivered. In our study, this was optimised by constantly checking and correcting this distance to compensate for movements, but it remains a technical difficulty that needs to be improved in treatment in the mouth.

ALA has advantages of a short period of cutaneous photosensitivity and the very superficial effect makes it safe. Excessive light exposure in the first 2 hours post sensitisation results in full thickness epithelial necrosis of skin. This heals without any significant scarring and realistically does little more than a very gentle chemical peel. It may yet have an application in this area. The depth of effect is inadequate for the treatment of significant tumour bulk, but dysplasia remains a reasonable target. Photofrin® has a much better depth of

effect – down to about 4mm. Healing is excellent, but treatment times are long and the three month photosensitivity period is inconvenient. Initial studies were hampered by technical problems – light of 635nm could only be delivered from a copper vapour or pumped dye laser, these machines were not easily portable and also unreliable. The advent of diode lasers has changed this perception and Photofrin® PDT has a potential clinical value. The most powerful sensitiser examined in this series of studies is Foscan®. The treatment times are short and produce a deep effect. There is a cost, however, and that is the potential for scar formation. Alteration in treatment parameters (see Chapter 8) might overcome some of these problems, but effective treatment of large tumours will result in damage to normal surrounding tissue until a truly selective effect can be demonstrated.

From these early studies, we went on to explore the clinical effects of PDT in different stages of oral precancer and cancer.

Chapter 8: Management of Dysplasia with Photodynamic Therapy

8.1 Introduction

Oral cancers can arise in clinically normal mucosa but are often preceded by premalignant lesions. The prevalence of such conditions in the general population may be up to 4.6%, (Axell 1987) with the rate of malignant transformation in oral epithelial dysplasia and leukoplakia with severe dysplasia quoted as 14% and 43%, respectively (Axell 1987, Lumerman et al 1995, Maerker and Burkhardt 1978). There are three principal areas of uncertainty associated with premalignancy. First, there are no widely accepted criteria for defining the presence and degree of dysplasia. Second, it is not clear which lesions require treatment, nor how aggressively such lesions should be treated (Speight et al 1993). Thirdly, many such patients exhibit a field change effect, so it may be necessary to treat large areas. In these circumstances, it may not be feasible to treat all affected areas at one time, so re-treatment may be necessary. Consequently, a modality with no cumulative toxicity is desirable.

Obviously, the first line of management is the elimination of risk factors such as smoking, drinking and Pan chewing. Active therapy includes surgical excision, topical cytotoxic therapy, systemic retinoid therapy, cryosurgery, or laser therapy, but none are entirely satisfactory or effective (Lamey 1993, Scully 1995),

Pre-malignant conditions of the oral cavity such as a leukoplakia and erythroplakia are clinical manifestations of increasing degrees of dysplasia. Dysplasia in the oral cavity is associated with an increased lifetime risk of malignant transformation. Histological grading of the lesion does not always provide a good prognostic factor for such transformation. By contrast Sudbo et al (2001), have shown that the DNA content can be used to predict the risk for malignant transformation in a wide range of oral precancerous lesions. While many clinicians would merely monitor mild dysplasia, few if any would be happy to monitor severely dysplastic changes. As dysplasia is caused by a number of genetic events, which are frequently widespread in the aerodigestive tract

where the tissue is clinically normal, even surgical resection of clinically dysplastic lesions has a high recurrence rate.

Management of oral dysplasia remains contentious. Clearly the conservative management of severe dysplasia amounts to supervised neglect, but at the same time, it is difficult to justify ablative treatment for a premalignant condition. There is a clear need for an intervention to treat or at least downstage dysplasia that is repeatable with no long-term side effects.

The studies reported here were carried out between 1992-1994 for Photofrin®, 1993-1995 for ALA and 1998-2004 for Foscan®. All cases were treated by me or under my supervision except for the Foscan® group that were all treated by me.

8.1.2 Photodynamic Therapy for Dysplasia

From the last chapter, it seems likely that PDT is potentially useful in dysplasia management. Photofrin® PDT produces a significant depth of effect without scarring. ALA has the potential advantage of a short period of photosensitivity, but has a fairly superficial effect. Foscan® PDT certainly has the deepest effect, however, some scarring might occur, especially when large areas are treated. In this chapter, I will describe studies of all three drugs in the management of dysplasia.

8.2 Treatment of Dysplasia with Photofrin®

The first drug to be investigated in the treatment of dysplasia was Photofrin®, a dihematoporphyrin ether (QLT, Vancouver, Canada). Although Photofrin® has been shown to be beneficial in certain clinical situations, (Gluckman 1991a) it has an ill-defined composition with poor tumour selectivity and causes prolonged cutaneous photosensitivity (6-12 weeks).

Based on experience gained with PDT, the management of dysplasia would seem an ideal treatment modality especially when applied to large areas. The nature of the photodynamic injury is such that healing should occur without any

of the side effects of radiotherapy or surgery leaving function and appearance preserved intact.

8.2.1 Materials and Methods

Following ethical approval, 6 patients were recruited to this study. All had histologically proven dysplasia – one had moderate dysplasia, the others had severe epithelial dysplasia of the oral cavity. All had refused or were unsuitable for conventional treatment. Prior to treatment, all had a standard haematological assessment which included a full blood count, urea and electrolytes.

8.2.2 Inclusion criteria

- Histologically proven dysplasia of the oral cavity
- All lesions were visible and accessible for treatment
- No upper limit on area
- Informed consent

8.2.3 Exclusion criteria

- Patients with history of porphyria
- Patients unable to give informed consent
- Patients with a life expectancy less than 3 months

8.2.4 Photosensitiser

Photofrin® was reconstituted from its freeze-dried formulation with 5% dextrose to give a concentration of 2.5mg/ml. Full instructions on light precautions were given prior to drug administration of Photofrin® 2 mg/kg body weight by slow intravenous infusion over 20 minutes. A drug light interval of 48 hours was used and all patients were treated under local anaesthesia as an outpatient.

8.2.5 Treatment and Results

Laser illumination was carried out using 630nm light from a copper vapour pumped dye laser (Cu25/DL 10 Oxford Lasers Ltd, Abingdon, UK). Light was delivered down a 400µm fibre which was mode scrambled to produce uniform illumination over the spot being treated. The laser power output was adjusted to give a fluence rate below 250mW/cm² to avoid thermal effects. The output was calibrated prior to each treatment and 50 –100J/cm² delivered to the tissue target. The outer edge of the treatment area was marked with crystal violet, and overlapping spots were used to treat the target area. This comprised the full extent of the lesion plus a 0.5cm safety margin of clinically normal mucosa. Where treatments were very long, an articulated arm (an ENT snake) was used to hold the fibre still. All treatment sites were recorded in the notes and follow up biopsies carried out when healing was complete regardless of clinical appearance.

Pt No	Area cm ²	Age/sex	SoD	SoP-M	Pre Rx	Light dose Jcm ²	EO 6-12 wks	LFU (mths)	LaFU (mths)
1	2	65/F	MD	LI	Nil	50	MiD		NC (139)
2	3	82/M	SED	LI	S & RT	100	PR	MD (4)	NC (130)
3	6	57/M	SED	L IT	E	100	CR	CR	CR (16)
4	3	51/M	SED	LI	Nil	100	CR	CR MD (4)	CR (12) Died Ca (72) Oesophagus
5	6 4 4	73/F	SED	R BM/ R&L Mand Alv	MS	100	PR	CO ₂ ERD	Recurrence (17) further PDT died (127)
6	9	45/F	SED	R Alv R BM	S	100	CR HK	CRats (26)	MCT (19) twf PDT

Table 8.1: Results of Photofrin® PDT in the Management of Dysplasia

Key: **Pt No**-patient number; **SoD**-severity of disease; **SoP-M**-site of pre-malignancy; **EO**-early outcome; **LFU**-longterm follow-up; **LaFU**-latest follow-up; **NC**-no change; **MiD**-mild dysplasia; **MD**-moderate dysplasia; **SED**-severe epithelial dysplasia; **MCT**-metachronous tumour; **twf**-treated with further; **S**-surgery; **MS**-multiple surgery; **E**-excision; **RT**-radiotherapy; **LI**-lower lip; **T**-tongue; **Alv**-alveolus; **BM**-buccal mucosa; **Mand**-mandibular; **HK**-hyperkeratosis **ERD**-excision for residual disease **CR**-complete response (clinical and biopsy) **ats**-at treated site; **PR**-partial response

Treatment was carried out without anaesthesia in 3 of the patients. Local anaesthesia was used in the remainder. Regional anaesthesia was used in preference to infiltration because of concerns about adrenaline in the anaesthetic solution. Theoretically, this can reduce the blood supply to the treatment site and if this results in a fall in oxygen tension could interfere with the PDT effect.

Following treatment, blistering was noted at 24 hours with swelling around the treated area. However, over the following 3 weeks, healing took place without any scarring. Some patients complained of temporary alteration in salivary flow – usually hyperptyalism – although this always settled by the time healing was complete. The results are shown in Table 8.1.

Apart from phototoxicity, there were no complications of drug administration and treatment was well tolerated by all patients.

8.2.6 Discussion - Photofrin®

The number of patients reported in this group is small. While only 3 patients had complete responses, 4 of the 6 have long term follow up. While it is not possible to make any statistically valid comments, it is interesting to note that patients with dysplasia of the lip did well. Although dysplasia was not completely eliminated, none of the three patients went on to develop invasive disease. One died of an oesophageal tumour 6 years later and the other two remain under long-term follow up after more than 10 years.

Of the other patients, one (5) who had a history of field change disease went on to develop multiple tumours and eventually died in 2003. She had approximately 20 different primary tumours plus widespread dysplasia during this time (in addition to diabetes and mitral valve disease). Only one patient was lost to follow up (6) who returned to the West Country. She remained convinced that it was not the PDT that helped her but Dead Sea Mud which she applied to her mouth.

Although we did not have any significant problems with phototoxicity, this group of patients were all carefully counselled and avoided sunlight for up to 3 months. This was something of a trial to some of the patients especially if the treatment was carried out during the summer. One interesting observation was that all patients who underwent Photofrin® PDT developed a very obvious suntan – this did not appear to be the case with the other drugs used in this chapter (ALA and Foscan®).

Photofrin® seems effective in the management of oral dysplasia. The clinical results of healing are excellent even when large areas are treated. Treatment was not without drawbacks however. Treatment times were long (1000s per spot) and the very large laser we used in these early studies was not always reliable and required a degree of medical physics input to generate a single photon. Of course this is not the case now that we have access to diode lasers that are portable and simply plug into a mains supply. The period of photosensitivity of up to 3 months was a problem for some patients and we did have some late phototoxicity problems although none of this group had serious problems.

8.3 5-Aminolevulinic Acid for Premalignant Lesions of the Oral Cavity

8.3.1 Background

ALA is unusual in that it is converted to a photoactive substance in vivo via the heme biosynthetic pathway. The systemic administration of ALA overcomes the feedback inhibition of ALA synthetase leading to the overproduction and accumulation of porphyrins pre-cursors to heme, in particular protoporphyrin IX (PPIX) (Pottier et al 1986). PPIX is the main photoactive substance produced following sensitization with ALA, (Pottier et al 1986, Kennedy et al 1992, Mustajoki et al 1992, Grant et al 1993b) and PDT using topical ALA has already been shown to be valuable in the treatment of basal cell carcinomas of the skin (Kennedy et al 1992). A major advantage of ALA is that, even after systemic administration, cutaneous photosensitivity only lasts 1 to 2 days (Mustajoki et al 1992). In addition, PDT-treated tissues heal remarkably well, making it feasible to treat extensive superficial lesions that often pose problems for conventional

management in the mouth (Grant et al 1993b). Further, in the authors' preclinical and clinical studies, PPIX has been shown to accumulate more in the epithelium than in the underlying muscle (Grant et al 1993b, Loh et al 1993b), with the consequent possibility of selective damage to this layer following PDT. The present study investigated the efficacy of PDT using ALA in the management of premalignant lesions in the mouth. The PDT effect produced by ALA would seem to be ideal for the treatment of dysplasia.

In previous studies performed in this centre, the effect of PDT using ALA was found to be very superficial, even with long treatment times (Grant et al 1993b, Loh et al 1993a, Regula et al 1995). The main concern was that the depth of effect would be insufficient if epithelial dysplasia is generated by an interaction between epithelium and deeper mucosal elements.

8.3.2 Materials and Methods

Twelve patients with histologically proven premalignant lesions of the mouth were sensitized with 60mg/kg ALA by mouth and treated with laser light at 628 nanometers (100 or 200J/cm²). These patients were treated with a gold vapour laser, elsewhere copper vapour pumped dye lasers and diode lasers have been used generating light of 630 – 635nm. The results were assessed macroscopically and microscopically. Biopsies were taken immediately prior to PDT for fluorescence studies, a few days after PDT to assess the depth of necrosis, when healing was complete, and up to 88 weeks later.

The study was approved by the hospital ethical committee, and all treatment was carried out with fully informed consent from the patients.

8.3.3 Inclusion and Exclusion Criteria

- See 8.2.2 and 8.2.3

ALA was supplied for this project as the hydrochloride in powder form by DUSA Pharmaceuticals (Tarrytown, New York). For clinical use, this was dissolved in orange juice and given by mouth in three separate doses of 20mg/kg at 0, 1,

and 2 hours (total dose, 60mg/kg) using the regimen established by Regula et al (1995). The light source used was a gold vapour laser giving red light at 628nm (Dynamic Light, Milton Keynes, UK). This was delivered to the patient using a single mode scrambled, 0.4mm flexible optical fibre with a bare flat-cleaved tip that gave a circular spot of light up to 2.5cm in diameter on the target tissue. The area to be treated was defined as the target lesion together with a margin of 5mm of surrounding normal tissue. Appropriate fibre positions and treatment times were calculated to give the desired light doses before treatment started, using one of the two dose regimens described below (Study Groups 1 and 2). Whenever possible, the entire lesion was treated from a single fibre position. However, due to the difficult shape of the mouth, this was often not practical, and up to seven different fibre positions had to be used in each patient. The power density was kept below 250mW/cm² to avoid thermal effects. As many of the total exposure times were quite long (up to 143 minutes), the fibre was positioned in a multi-jointed arm to keep its tip in the correct place over the area to be treated. Patients were given systemic analgesia and topical anaesthetic with occasional sedation if necessary. Injected local anaesthetic was limited to the site of biopsy.

Study Group 1 (7 patients) received a total fluence of 100J/cm² in two equal fractions, with a minimum of one 5-minute interval between fractions. With the long treatment times required, it was sometimes uncomfortable for patients to keep their mouth open continuously, so in these cases there were further breaks during treatment.

Study Group 2 (5 patients) received a total light fluence of 200J/cm² in two equal fractions. The first fraction was delivered 2.5 hours after the first dose of ALA, and the second was delivered at 4 hours.

The depth of necrosis in these two groups was not statistically significantly different, so for the purposes of clinical outcome were treated as one group.

Patients were kept in a dark room for 24 hours after receiving ALA. Routine haematologic and biochemical investigations were performed before and 1 to 2 days after ALA ingestion (longer if any abnormalities were found).

8.3.4 Results

Complete epithelial necrosis was present in all cases. All 12 patients showed improvement (repeat biopsy was normal or less dysplastic) and the treated areas healed without scarring. No patient had cutaneous photosensitivity for longer than 2 days. Patients reported experiences ranging from mild discomfort to severe pain during the time of irradiation, in some cases requiring more analgesia than had been anticipated. Significant discomfort was experienced by 6 of 12 patients. Nausea and vomiting was experienced by four. Nausea was often an early feature presenting during the period of drug administration, whereas vomiting started several hours after ALA ingestion and resolved by 24 hours. Only one patient had cutaneous photosensitivity lasting longer than 24 hours, but this resolved by 48 hours. After PDT, the treated area became inflamed within a few hours. This was followed by sloughing of the superficial layers after 1 to 2 days, leaving a shallow ulcer. Oral analgesics were usually required from the first or second day after PDT for approximately 1 week. Excellent healing was found in all areas irrespective of the size of the original lesion, although larger lesions often took longer to heal (3 to 5 weeks). There was no evidence of scarring in those lesions that had not undergone previous surgery, other than at the site where biopsy specimens were taken (Fig1). Once healed, patients did not perceive any changes in function or sensation within the oral cavity.

8.3.5 Clinical Outcome

All 12 patients were reassessed when healing was complete, and 9 underwent further biopsies at this stage (2 to 12 weeks after PDT). Long term follow-up data were available on all 12 patients, 10 of whom underwent a late follow-up biopsy. Those who refused repeat biopsies usually did so because the treated areas looked macroscopically normal. Details of the specimens taken and the histologic finding are given in Tables 8.2 and 8.3.

All seven patients with moderate dysplasia improved after treatment, i.e., the mucosa became normal or only mildly dysplastic. However, in one case that only showed hyperplasia just after healing, moderate dysplasia was seen again

48 weeks later (Table 8.2). In four of five patients with severe dysplasia, there was no evidence of residual disease after treatment with 36 to 78 weeks of follow-up, although one refused biopsy at the long term follow-up. In the other patient, moderate dysplasia was seen at early and late follow-up (Table 8.3). It is notable that in three of the patients with regression but not clearance of dysplasia (Patients 1, 3 and 9), there was no macroscopic evidence of disease, the mucosal surfaces appearing clinically normal once the PDT-treated area had healed.

One patient initially included in the SED group underwent a pre-PDT biopsy that showed just severe dysplasia. It was only after ALA PDT, when 88% of the mucosal lesion had cleared, that a biopsy was performed on the residual lesion and it was found to be microinvasive SCC with changes extending down the salivary ducts (Fig.12). This suggested that PDT using ALA was able to deal with the severe dysplasia in this case but not the deeper invasive disease. As this patient was not suitable for surgery, further PDT was carried out using Photofrin® with no evidence of tumour at 11 months follow-up.

No changes were noted in the haematologic indices, although elevation of liver enzymes was observed in nine cases compared with baseline values obtained before ALA ingestion. The enzyme most commonly affected was aspartate transaminase (AST). The AST level rose above the normal range in only five cases, (maximum: 3.5 times the upper limit of normal), with bilirubin elevated in only two. Monitoring of liver function was only continued more than 2 days after ALA ingestion in cases with elevated AST levels. In all cases this was asymptomatic and returned to normal levels within 10 days, with the exception of one patient in whom liver enzymes were elevated for 30 days – this patient had a history of excess alcohol intake. One patient developed a pruritic rash 1 week after treatment, which on biopsy was diagnosed as a cutaneous lichenoid reaction. This gradually resolved uneventfully over the following 3 weeks.

Pt no.	Age/sex	Site	Previous Treatment	Histology healed/wk	Size of lesion before/Healed	Histology longest follow-up/wk
1	73/M	BM	Nil	Mild (5)	2.6cm ² /1.7cm ²	Mild (40)
2	69M	FM	CO ₂ laser	Normal (5)	6.2cm ² /0cm ²	Normal (32)
3	52M	FM	Nil	Mild (4)	7.1cm ² /0cm ²	N (28)
4	79M	T	Nil	Mild (6)	3.1cm ² /0cm ²	Mild *56)
5	67/M	BM	Nil	Normal (5) [†]	1.8cm ² /0cm ²	Moderate (48)
6	69/F	T	Nil	Mild (5)	3.1cm ² /0.8cm ²	Mild (64)
7	46/M	A	CO ₂ laser	N (5)	1.0cm ² /0cm ²	Normal (64)

BM: buccal mucosa; FM: floor of mouth; N: no clinical disease macroscopically, but biopsy refused; Normal:histologically proven no dysplasia;T: tongue; NM: not measurable; A: alveolus.

*Inflammatory changes extend through full thickness of biopsy specimen.

[†]Hyperplasia seen, but no dysplasia.

Full-thickness epithelial necrosis and sloughing present in all cases.

Patients 1-4 received 100J/cm²; patients 5-7, received 200J/cm².

Table 8.2: Clinical Outcome of Patients with Moderate Dysplasia

Pt no.	Age/sex	Site	Previous Treatment	Histology healed/wk	Size of lesion before/Healed	Histology longest follow-up/wk
8	51/M	FM	Nil	Normal (5)	6.3 cm ² /0cm ²	Normal (6)
9	75/M	BM	CO ₂ laser	Moderate (2)	7.1cm ² /0cm ²	Moderate (54)
10	70M	BM/A	Nil	Normal (8) [*]	8.8cm ² /0cm ²	N (36)
11	49M	T	CO ₂ laser	N (12)	8.0cm ² /0cm ²	Normal (78)
12	59/F	T	CO ₂ laser	N (12)	4.8cm ² /0cm ²	Normal (76)

FM: floor of mouth; BM: buccal mucosa; A: alveolus; N: no clinical disease macroscopically, but biopsy refused; Normal:histologically proven no dysplasiaT: T: tongue.

*Hyperplasia seen, but no dysplasia.

Full-thickness epithelial necrosis and sloughing present in all cases.

Patients 8-10 received 100J/cm²; patients 11 and 12 received 200J/cm².

Table 8.3: Clinical Outcome of Patients with Severe Dysplasia

8.3.6 Discussion - ALA

ALA PDT has considerable attractions for treating premalignant and early malignant lesions of the mouth. It is relatively easy to apply, there is no cumulative toxicity, and healing is superior to other forms of local tissue destruction as the epithelium and lamina propria heal mainly with regeneration and only necrosed muscle is likely to scar.

Reviewing results in the present ALA series, patients with dysplasia did well. All 12 patients showed improvement after PDT. Ten patients (83%) showed clinically (macroscopic) normal mucosa when healing was complete, although of the nine that underwent biopsy at this time, one showed moderate and four showed mild dysplasia. It was of some concern that persistent dysplasia was seen in lesions that macroscopically had reverted to normal, although the changes in these areas were never more than moderate dysplasia. Even patients in whom the biopsy specimen was normal after PDT cannot be regarded as cured, but the risk of malignancy should have been reduced and they can be given PDT with ALA again if necessary for regression. One of our patients with moderate dysplasia had a normal biopsy specimen after PDT but subsequently regressed to moderate dysplasia again on check biopsy 1 year later. This is not surprising as one is dealing with a field cancerisation process and premalignant lesions of the mouth have a high recurrence rate after all forms of treatment. Nevertheless, the risks are lower with milder degrees of dysplasia, and oral leukoplakia associated with mild dysplasia only has a 3% risk of malignant transformation (6). PDT seems to down regulate the severity of dysplasia in these patients; as it can be repeated if necessary, it is reasonable to hope that their disease can be kept under control for many years with minimal morbidity.

The maximum depth of necrosis (Chapter 7) was 1.3mm, comparable to the results found by Regula et al (1995), in gastrointestinal tumours. And it would be clearly preferable if the PDT with ALA could produce a greater depth of necrosis. Techniques for achieving this are outlined in Chapter 7.

The convenience of the short cutaneous photosensitivity using ALA means that PDT can be repeated at short intervals if necessary.

8.4 Foscan® Photodynamic Therapy in the Treatment of Severe Epithelial Dysplasia

The main disadvantage of ALA treatment is the limited depth of effect (1mm) which makes it inadequate in the presence of co-existing early invasive disease. Added to that are prolonged treatment times up to 1000 seconds a spot. Photofrin® PDT was used as well with a slightly better depth of effect (5mm) but prolonged skin photosensitivity up to 6 weeks. Meta tetrahydroxyphenyl chlorin (mTHPC, Foscan®®, Biolitec) has been shown to be an effective photosensitiser in treating invasive disease, but with conventional doses of 0.15mg/kg produces excessive tissue necrosis with scarring (Hopper). Kubler et al (2003) have shown that it is possible to treat skin cancer with a reduced drug dose and light doses and still produce a good depth of necrosis. In this study we aim to show that it is possible to treat severe epithelial dysplasia (SED) in the mouth using low dose Foscan® PDT.

8.4.1 Introduction

Foscan® PDT can effectively ablate disease of the oral mucosa down to a depth of up to 1cm. In the last chapter we demonstrated that the cost one bears with this is some degree of scarring with tethering of sensitive tissues such as the tongue. However, it is possible to get a reasonable depth and effect using Foscan® PDT by reducing either the drug or the light dose. We, therefore, carried out an incremental study using different light doses of 5 up to 15J/cm² and a drug dose of 0.1mg/Kg. This is by no means a comprehensive study but does go some way to demonstrate the ability one has to modify the side effect profile by changing the treatment parameters.

8.4.2 Materials and Methods

22 patients with histologically proven SED were recruited for this study. They underwent surface illumination PDT using Foscan® injected 96 hours prior to

treatment with a diode laser. The study was approved by the ethics committee of University College London Hospitals and patients were fully informed and consented about the procedure. The male to female ratio was 1:1 with a mean age of 65 years (age range 34 - 98yrs). Mean follow up was 30 months (2-68m) while mean disease free interval was 12.8 months (2-63m). Mean disease free interval was defined as the time between treatment and the diagnosis of a new lesion.

8.4.3 Inclusion and Exclusion Criteria

- See 8.2.2 and 8.2.3

8.4.4 Photodynamic therapy

Foscan® was supplied in 5ml vials with a concentration of 4mg/ml. The intravenous dose of 0.1mg/kg was administered to the patient 96 hours prior to the treatment. Due to systemic skin photosensitivity after the iv injection of the photosensitiser, every patient was instructed on the need to avoid direct sun exposure for 3 weeks and was given written light precautions. Patients were allowed exposure up to 100 lux of light for the first day (60W light bulb) and increased by increments of 100 lux per day for the following three weeks. A Biolitec® diode laser was used emitting red light at 652nm wavelength.

45% (10/22) of the patients had their treatment carried out under local anaesthetic. 55% (12/22) had a general anaesthetic in order to improve access. Treatment times ranged between 50-600 seconds with a mean of 202 seconds. The treatment area was defined as the lesion with 1cm margin of normal tissue. This area was marked with a marker pen to allow repositioning or resizing of the irradiated spot made necessary by patient movement. The average number of spots per patient was 1.8 (range 1-6). The average size of the spot was 2.5cm with a range between 1.5-4cm. 27 sites were treated, 77% of patients had one site treated while 23% had 2 or more sites treated. 48% of lesions were on the tongue. Other sites included palate, floor of mouth, alveolar and buccal mucosa. Three different light doses were used. The first three patients were

treated with a light dose of $5\text{J}/\text{cm}^2$. The next 14 patients were treated with $10\text{J}/\text{cm}^2$. The last 5 patients were treated with $15\text{J}/\text{cm}^2$.

8.4.5 Results

The first three patients were treated with a light dose of $5\text{J}/\text{cm}^2$ (table 8.4). All 3 patients went on to develop further areas of SED which required further treatment. 2 out of 3 patients went on to develop squamous cell carcinoma requiring major surgical resection +/- radiotherapy. Based on these results we decided to abandon the $5\text{J}/\text{cm}^2$ light dose and increased it to $10\text{J}/\text{cm}^2$. 14 patients were treated using this light dose. 4 patients showed complete response (CR) and are disease free with a mean follow up of 18 months. 10 patients showed partial response (PR). 3/10 went on to develop SCC while the other 7 developed further areas of mild/moderate/ severe epithelial dysplasia. 1 developed another primary in the larynx and the oesophagus. 1 died of unrelated disease. 9/10 patients required further treatment for their disease. In total 11/14 patients are now disease free. 12/14 patients are alive. Mean disease free interval is 13 months. Median disease free interval is 7.5 months. The latest protocol involves using $15\text{J}/\text{cm}^2$. Five patients were treated with 100% complete response. Mean follow up of 9.6 months.

The main complications noted were pain and swelling. All patients required analgesics in the early ulcerative phase, with oral opiates prescribed for 1-2 weeks post PDT. No cutaneous photosensitivity was noted. It was noted that the site of iv injection of Foscan® was the most photosensitised in comparison to the rest of the body and extra care and light precautions were needed for at least six weeks. The procedure was well tolerated by the patients under local anaesthetic. No scarring was noted in the histological samples or clinically at sites of treatment. The treatment times ranged between 50 and 600 seconds with a mean of 262 seconds which is much quicker than PDT with other photosensitisers.

Patient	Age/ Sex	Site	Prev Rx	Light Dose J/cm ²	DFI (m)	Further Rx	Outcome
1	65/M	P	L	5	15	S, RT	NCD
2	60/F	T	ALA	5	17	S, PDT, ALA	Mild ED
3	70/F	T	Nil	5	14	L	Mod ED
4	57/M	T	SCL	10	4	L, PDT	Mild ED
5	42/F	T	Nil	10	23		NCD
6	78/M	T, P, FoM	S	10	1	PDT	SCC (RIP)
7	73/M	T	Nil	10	63		SED
8	51/M	T, FoM	Nil	10	10	L	Mild ED
9	54/M	T	RT, C	10	31		NCD (2 nd L)
10	73/M	T	ALA	10	5	L	NCD
11	98/M	AM	S	10	3	S, L	Mild ED (SED out)
12	75/F	T	Nil	10	20		NCD
13	64/F	T	Nil	10	2	L	NCD
14	80/F	P	S, LPDT	10	10		NCD
15	74/F	P, AM	Nil	10	3	S, L	NCD
16	67/M	BM	S, PDT, ALA, L	10	4	PDT, ALA, L	NCD
17	34/F	T	L	10	12	ALA	NCD (SED out)
18	43/F	T	Nil	15	13		NCD
19	83/M	BM, P	S	15	11	L	NCD
20	68/M	P	Nil	15	7		NCD
21	62/F	P	S	15	10		NCD
22	62/F	P	Nil	15	7		NCD

Table 8.4: Treatment results - 22 patients

Key: T-Tongue, P-Palate, FoM-Floor of Mouth, AM-Alveolar Mucosa, BM-Buccal Mucosa, DFI-Disease Free Interval, Rx-Treatment, S-Surgery; C-Chemotherapy; L-CO₂ Laser; RT-Radiotherapy; PDT-Foscan® PDT; ALA-Photodynamic therapy with 5-aminolaevulinic acid; NCD-No clinical disease; Mild ED-Mild Epithelial Dysplasia; Mod ED-Moderate Epithelial Dysplasia; SED-Severe Epithelial Dysplasia; 2nd L-Second primary in larynx.

8.4.6 Discussion - Foscan®

The results of this study were slightly less predictable than one would have at first thought. Despite the fact that clinical responses were quite impressive at 10J/cm² and 0.1mg/Kg this did not translate into an acceptably high 'cure' rate. We know from other studies that 20J/cm² and 0.15mg/Kg (the dose usually used for treatment of early invasive squamous cancer – see Chapter 9) provides a suitable depth effect for treatment of this condition. However, it would seem that to safely ablate severely dysplastic disease (which may indeed carry foci of microinvasive disease) 15J/cm² and 0.1mg/Kg would seem to be a safe dose. This is indeed borne out by parallel studies by Kübler et al (2003) in Cologne.

"Field cancerisation", a concept introduced by Slaughter et al in 1953, described the potential for patients to develop multiple primary cancers from independent premalignant foci. This is well represented in our cohort of patients. It was interesting to note that 55% of our patients had previous treatments for either SCC or SED including one or more modalities of treatment. A further 8/18 developed further areas of SCC or dysplasia following their PDT treatment. This may represent sample bias, and perhaps treatment should have been confined to virgin cases of dysplasia. By including patients who had developed dysplasia after previous treatment, we had already selected a group who were likely to have widespread genetic damage, even though this might not be clinically apparent. It would seem likely that this group had an increased risk of dysplasia and metachronous tumours.

Foscan® PDT provides an alternative approach to treating SED. It is not only well tolerated but also simple and quick to execute. There is no cumulative toxicity and as such if response is incomplete areas can be retreated with PDT. Areas heal much better than other forms of local tissue destruction as the epithelium and lamina propria regenerate. At the reduced dose of Foscan® at 0.1mg/kg, minimal scarring if any was noted on the post PDT biopsies. 5J/cm² and 10J/cm² light doses are not adequate to treat SED and thus the recommendation is to treat SED with 15J/cm². Visible dysplasia should be treated with a wider margin to improve outcome. 22% of patients went on to develop SCC, which corresponds to the rate of malignant transformation quoted

in the literature (malignant transformation of epithelial dysplasia quoted as 14% oral epithelial dysplasia and leukoplakia with severe dysplasia 43%, Axell 1987, Lumerman et al 1995, Maerker and Burkhardt 1978).

8.5 Conclusions

Photofrin® is useful in the management of selected cases of widespread dysplasia. However, the need for a better drug with a shorter treatment time and a shorter period of photosensitivity is obvious.

ALA produces consistent epithelial necrosis with excellent healing in dysplasia of the mouth and is a simple and effective way of managing patients. There are obvious limitations.

Foscan® PDT provides a very good option to treat SED in cases of wide field change with few complications. There are small but definite benefits from changing the drug light prescription for the management of pre-malignant disease which seems to reduce morbidity in terms of scarring and photosensitivity. In this study we have not addressed the other variable which is the drug light interval and recent work in Russia would suggest that reduction of this time period would allow an even greater reduction in the amount of drug used in the photosensitisation and also the amount of light to precipitate a photochemical effect. Having found a level at which dysplastic tissue can be treated effectively with a reduced drug and light dose has two major benefits. Firstly the period of photosensitivity is reduced. By reducing the drug from 0.15mg to 0.1mg/Kg the need for avoidance of artificial light is greatly reduced. There is obviously a need to return to normal sunlight exposure in a controlled fashion but the photosensitive period is reduced by approximately four days by using this cocktail. By reducing the light dose somewhat the amount of pain and collateral damage from reflected light also seems reduced. This is a rather empirical finding and has not been quantified in this study.

8.6 Summary

PDT with a variety of sensitisers is effective treatment of oral dysplasia, although the three drugs described have their advantages and disadvantages (table 8.5). ALA has the shortest period of photosensitivity but has a limited depth of effect. Photofrin® has a better depth of effect, but treatment times, along with ALA are long and there is also the added problem of prolonged photosensitivity. Foscan® can be used with a modified dose regime and seems to produce a consistent effect with a 2 week photosensitivity period. The great advantage is that treatment times are very short. It is also possible that alterations in the drug light interval will allow an even shorter period of photosensitivity. Clearly, careful case selection is required but at the present time, Foscan® would seem to be the drug of choice for SED, especially when microinvasion is suspected, but not found in the biopsy specimen. ALA can also be useful, but careful evaluation of each case is necessary as the effect is so superficial.

Drug	Dose	DLI	Wavelength	Depth of effect	Photosensitivity period	Treatment time /spot
Photofrin®	2mg/kg	48hrs	630nm	5mms	6 weeks +	1,000s
ALA	30 (IV) 60 (PO)mg/kg	2 – 5 hrs	628-635nm	1-1.5mms	24 - 48hrs	1,000s
Foscan®	0.1mg/kg	96hrs	652nm	5 - 7 mms	2 weeks	150s

Table 8.5:Treatment parameters and depth of effect in dysplasia management

Chapter 9: Photodynamic Therapy in the Management of Early (T1) Squamous Cell Carcinoma of the Oral Cavity

9.1 Introduction

The mainstays of treatment for early disease are surgery and radiotherapy, either alone or in combination. The results of treatment vary considerably with the site and stage of the primary lesion. Initial local control is achieved in 67% to 95% of T₁ tumours dropping to 50% to 81% with T₂ disease (Munro 1995, Hong et al 1990).

The probability of local control at two years for patients with T₁ or T₂ SCC of the tongue is 76% to 79% (Munro 1995, Hong et al 1990).

The specialised functions of the oral cavity and face mean that the surgical treatment even of small volume tumours can cause functional and aesthetic impairment leading to withdrawal and social isolation. Treatment for T₂ tumours may involve major surgical ablation and reconstruction in certain anatomical sub-sites. Radiotherapy is used extensively because it has less impact on structure and function, but it is not without problems. It is generally given as a single treatment course requiring daily hospital attendance for four to six weeks. It has complications caused by damage to tissue vasculature, which impairs wound healing, and this further complicates surgical salvage after failed radiotherapy. In the oral cavity, there is distressing xerostomia, which is permanent and almost impossible to treat (Fein et al 1994). Late complications of osteoradionecrosis, soft tissue necrosis, spinal cord myelitis, vascular damage and endoarteritis are also recognised (Fein et al 1994). In the younger population or in those with field change disease there is the potential for radiation-induced tumours (van der Laan et al 1995).

Most depressingly, there seems to have been little improvement in overall survival, which has not changed in the last 30 years (Blair 1994). Treatment strategies therefore should be targeted at the maintenance of aesthetics, function and quality of life, in addition to survival.

Even with small tumours there is an annual risk of 10% to 30% of developing metachronous tumours. Management of the second primary or recurrent tumour in previously treated areas is a difficult problem (Carr et al 1989, Gluckman et al 1983). There is clearly a need for alternative treatments that are repeatable in the field of previous radiotherapy without the tissue ablation associated with surgery. PDT may provide such a treatment. In this chapter, experiences with three different photosensitisers are described in the chronological order in which they were used. Broadly speaking, the Photofrin® was used from 1991 to 1994, ALA was studied from 1993 to 1994 and Foscan® from 1994 to 2000 with the latest follow-up at January 2005. All the studies described here underwent internal peer review with myself as principle investigator. All patients were under my sole care and the treatments were carried out by research fellows Mr W Grant and Ms K Fan under my supervision or by myself.

9.2 Photofrin® PDT

This was the first drug to be used in widespread clinical practice (see section 3.3). Results with porphyrins or their derivatives with a light activating wavelength of 630nm are encouraging (see section 7.2), but the exact treatment parameters in the published works are variable and often impossible to compare (Li et al 1990, Gluckman 1991b, Grant et al 1993b, Biel 1998). Initial studies using the drug were reported in advanced disease, however, it seems much more logical to use this treatment in early disease where the total volume of disease can be treated. We had demonstrated that in the clinical setting, PDT damage could be inflicted on normal oral tissues with excellent healing, probably as a result of the preservation of collagen and elastin.

9.2.1 Aim

The aim of this study was to establish the efficacy of PDT treatment using the commercially available di-haematoporphyrin ether/ester mixture (Photofrin® QLT Vancouver, Canada) in the treatment of early (T1) squamous cell carcinoma of the oral cavity.

9.2.2 Materials and Methods

Four patients with histologically proven early invasive squamous cell carcinomas of the oral cavity, were included in the study group. (See Table 9.1). All tumours were diagnosed as Stage 1 ($T_1N_0N_0$) oral cancers and no deep extension to underlying muscle or bone was suggested by the clinical, histological or radiological findings. No patient had received radiotherapy. Informed consent was obtained in each case.

Photofrin® at a dose of 2mg/kg was given by intravenous infusion 48 hours prior to surface illumination of the tumour. A copper vapour pumped dye laser was used to deliver monochromatic red light at 630nm. An optical fibre with a microlens affixed ensured homogeneity of the illuminating light spot, and a multi-jointed articulated arm was used to facilitate positioning of the fibre in three planes.

All four patients were treated in a single session with a single spot of light. A light dose of 50 to 100J/cm² was delivered to each patient. Power densities were kept below 150 mW/cm² to avoid any possibility of thermal injury, and no patient reported any sensation of heat, although some described various sensations such as tingling and tickling during light exposure. Patients were counselled to avoid direct sunlight following sensitisation. Patients were prescribed analgesics and benzydamine local analgesic or anti-inflammatory spray.

Lesions were photographed and treatment areas were recorded diagrammatically on referral. Following treatment patients were reviewed and photographed twice in the week after PDT and then approximately weekly until healed, and thereafter every two to four weeks.

Three patients had biopsies of regenerated clinically normal mucosa, at sites where invasive carcinoma had been previously diagnosed. Each was taken more than three months after PDT.

9.2.3 Results

All treatments were performed without local anaesthetic or sedation and were satisfactorily tolerated by each patient, on an outpatient basis. During the three to four days after PDT the treated areas underwent obvious surface necrosis with the development of a superficial slough, and local oedema. While no pain was associated with the treatment itself, all patients reported some degree of pain over the following days, usually lasting about one week, but this was effectively relieved by oral analgesia. Eating tended to exacerbate pain and a sloppy diet was advised and found to help. Treated areas took between three and five weeks to heal fully, depending on the size of area treated.

At assessment following healing, six to eight weeks after PDT, all four patients showed a complete response to treatment. The healed mucosa was of normal colour with no evidence of leukoplakia or erythroplakia (Figure 9.1a and 9.1b).

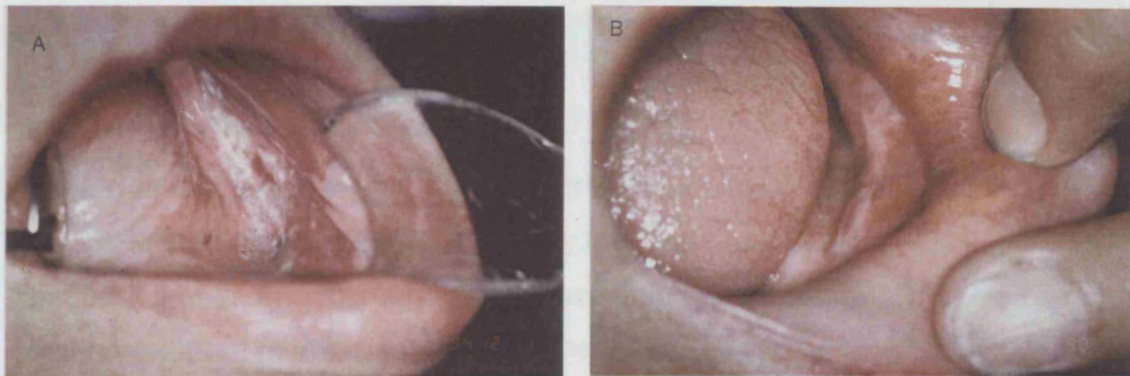


Fig 9.1: Early SCC left cheek before and after Photofrin® PDT

This patient had a retromolar trigone area treated, but was excluded from the study because of the extensive sublingual keratosis.

Patient No	Age/Sex	Disease Stage	Site	Previous Treatment	Light Dose J/cm ²	Outcome 6-12 wks	Longest FU (mths)
1	60/F	T1	Tongue	Surgery	100	Mild dys	CR 36
2	33/F	T1	Tongue	Nil	100	CR	CR 48
3	40/M	T1	Lip	Surgery	50	CR	CR 60
4	62/M	T1	FOM	ALA PDT	100	CR	CR 16

Table 9.1: Clinical responses with Photofrin® PDT

Key: FOM-Floor of mouth; CR-Complete response

9.2.4 Discussion

From this very small sample, the results of treatment were really quite encouraging. However, the difficulties of unreliable lasers, long treatment times and long periods of photosensitivity led to the abandonment of this drug in my clinical practice.

9.3 ALA PDT

There was much excitement when we first started using ALA for photodynamic therapy in the oral cavity. Unfortunately the depth of effect as described in chapter 7 would tend to suggest that it would not have been an ideal approach for the treatment of invasive cancer. The initial thought was that if light of 630nm penetrated up to 5mm that while surrounding tissues may be left undamaged, any tumour of epithelial origin would be susceptible to the PDT effect. Unfortunately this was not borne out in the clinical study and while the treatment was very well tolerated by all patients without any side effects of scarring the clinical results soon resulted in the approach being stopped.

9.3.1 Materials and Methods

Six patients with histologically proven SCC lesions of the mouth were sensitized with 60mg/kg ALA by mouth and treated with laser light at 628nm (100 or 200J/cm²). The results were assessed macroscopically and microscopically.

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Biopsies were taken prior to PDT, a few days after PDT to assess the depth of necrosis, when healing was complete, and up to 88 weeks later.

The study was approved by the hospital ethical committee, and all treatment was carried out with fully informed consent from the patients. The supply of ALA, preparation and treatment methods are the same as outlined in Chapter 8. After treatment, patients were kept in a dark room for 24 hours after receiving ALA. Routine haematologic and biochemical investigations were performed before and 1 to 2 days after ALA ingestion (longer if any abnormalities were found).

9.3.2 Results

Following treatment, all patients showed superficial ulceration at the site of illumination. There was no evidence of a selective tumour effect, but there was also no evidence of damage to deeper structures such as muscle. Although the treatment itself was often painful (even with administration of copious local anaesthetic), the post treatment recovery was quite uneventful with only minor analgesics being required. In the first two patients (1,2 Table 9.2) healing was complete at 2 weeks and the results were very encouraging – one had a complete response that has been sustained for almost 10 years. The second patient, although she had persistent mild epithelial dysplasia on post treatment biopsy remained well until she died of a stroke over 5 years later. The results with the other patients were less satisfactory and all had to go on to further treatment. This was typically demonstrated by case no 5 which is illustrated in figure 9.2.



Fig 9.2: a) Before PDT, with severely dysplastic lesion; b) Four weeks after PDT with ALA, residual patch found to be microinvasive disease.

Of the partial responders with persistent invasive disease, it was necessary to go on to further treatment. Of course this remains one of the most useful aspects of PDT treatment – that it does not adversely affect other treatments such as surgery and radiotherapy. However, this result might have been predicted from the studies conducted in Chapter 7 where the depth of effect was shown to be a maximum of 1 – 1.5mms.

Age/Sex	Site	Previous Treatment	Pre Treatment	Post Treatment	Histology	FU (mths)
1 52/F	FOM	CO ₂ laser	1.8cm ²	No clinical disease	Normal	100
2 85/F	P	Surgery	4.6cm ²	0.8cm ²	Mild dysplasia	67 (RIP CVA)

A further four patients were treated but all had invasive squamous cell carcinoma and had progressive disease requiring further treatment hence not evaluable except for safety data. Patients 3 4 and 6 went on to surgery and 5 gained a complete response with Photofrin®

Age/Sex	Site	Previous Treatment	Pre-Treatment	Post-Treatment	Histology/longest FU (mths)
3 87/F	T	Surgery	4cm ²	0cm ²	NA
4 81/F	A	Mandibular resection	16.7cm ²	0.6cm ²	NA
5 62/M	FOM	Nil	8cm ²	1cm ²	NA [‡]
6 65/F	BM, RM, AP	Surgery	8.5cm ²	Unchanged	NA

Table 9.2: Clinical Response with ALA PDT

Key: T-Tongue; A-Alveolar; FOM-Floor of Mouth; BM-Buccal Mucosa; RM-Retro Molar; P- Palate; AP-Anterior Palate

[‡]Subsequent photodynamic therapy with a different sensitiser.

Patients 3 - 6 received 100J/cm²; patients 1 and 2 received 200J/cm².

9.3.3 Discussion

Despite the obvious attractions of ALA in the treatment of invasive disease – a short drug light interval, a short period of photosensitivity and excellent post treatment healing, the superficial effect made it impossible to continue this therapy for invasive disease.

9.4 Foscan® PDT

Foscan®, is a pure, synthetic chlorin. It has the advantages of short light activation times with a depth of effect possibly to as much as a centimetre when activated with red light at 652nm (Berenbaum et al 1986b) and photosensitivity is limited to two to three weeks (Ris et al 1991). Several pilot studies have been published suggesting Foscan® PDT is a useful treatment in early oral cancer (Dilkes et al 1995, Savary et al 1997, Fan et al 1997). In this section the results of a multicentre study are presented for which the author was writing committee chairman. Also, the results of the patients enrolled into the study from the authors practice are included as the follow up is now several years.

9.4.1 Study Aims

To determine the response rate of early SCC (T_{is} , T_1 and T_2 up to a maximum diameter of 2.5 cm, maximum depth of 0.5 cm) to single dose Foscan® PDT and to assess the tolerability and safety of the treatment.

9.4.2 Study Design

This was a multi-centre, open-label, single group Phase IIb study into the effect of Foscan® PDT on patients with a histologically confirmed primary SCC of the oral cavity. This description covers the 114 patients recruited from all centres. Results from the 12 patients recruited from UCLH have been extracted from the main body of the data and summarised in section 9.48.

Foscan® (0.15mg/kg body weight) was administered by intravenous injection 96 hours before tumour illumination with 20J/cm² of laser light of 652nm wavelength (Diomed, Applied Optronics Corporation (AOC), Laserscope) delivered by superficial illumination via a standardised optical fibre and microlens diffuser (Medlight, Pioneer).

Following treatment, patients were observed on Days 1, 2 and 7 and Weeks 2, 4, 6, 8 and 12. The response at the tumour site was assessed according to standard WHO criteria (WHO 1979). Biopsies were taken where possible 12

weeks after PDT. Assessments of safety variables were also made during the study.

9.4.3 Materials and Methods

Local ethics committee approval was obtained for the study and each patient was required to give informed written consent. Excluding the 7 protocol violators, 69 male and 45 female patients were enrolled to the study with a mean age of 64 years (Table 9.3). The 7 protocol violators were withdrawn for a variety of reasons (Table 9.4).

Demographic characteristics for all patients in the 12 week study	
N = 114	
Mean age (range)	64 (30-99)
Male	69 (60%)
Female	45 (40%)

Table 9.3: Demographic characteristics

7 patients recruited to the study were withdrawn at an early stage due to protocol violations (Table 9.4).

Reason for withdrawal, protocol violations	
N = 7/121	
2	Laser failure
1	Miscalculation of light dose
1	Adjunctive treatment used (CO ₂ laser) before PDT treatment
2	Tumour found to be deeper than 5mm
1	Illumination delayed due to intercurrent patient illness

Table 9.4: Withdrawals due to protocol violation

Male or non-pregnant, non-lactating female patients aged 18 years and over who had primary SCC of the lip, oral cavity, oropharynx or hypopharynx; a Karnofsky status of ≥ 70 ; and who presented with a single discrete locally treatable T_{is} (3), T_1 (94), T_2 (17), N_0 , M_0 tumour (≤ 2.5 cm diameter, ≤ 0.5 cm estimated depth), which was clearly visible and accessible for illumination were entered into the study. The maximum diameter of 2.5cm was selected to enable treatment with a single spot illumination of the whole tumour with an adequate margin of normal tissue. Tumour depth was estimated by clinical palpation and standard imaging techniques (CT, MR ultrasound). The maximum depth was limited to 0.5cm to ensure the treated tumour volume was consistent with the known depth of penetration of Foscan® PDT (Table 9.4).

Exclusion criteria: Patients who had undergone (or were scheduled for) radiotherapy or neck dissection in continuity, or were receiving chemotherapy; and patients with active hepatic or renal disease, or any disease caused or exacerbated by light were excluded from the study.

Disease assessment for patients entering the study N = 114	
Site of tumour	
Lip	23 (19%)
Tongue	22 (19%)
Gum	2 (2%)
Floor of mouth	44 (38%)
Palate	7 (6%)
Buccal mucosa	12 (11%)
Other	4 (3%)
Tumour TNM class	
T _{is}	3 (3%)
T ₁	94 (83%)
T ₂	17 (14%)
N ₀	113 (99%)
N _x	1 (1%)
M ₀	114 (100%)
Tumour depth (mm)*	
Mean	3.0
Range	0.1 - 5.0
Tumour area (cm²)	
Mean	2.1
Range	0.1 - 8.1
Tumour largest diameter (cm):	
Mean	1.7
Range	0.5 - 2.5

Table 9.5: Disease assessment

9.4.4 Photodynamic Therapy Regimen

Foscan® was administered at a dose of 0.15mg/kg body weight by slow intravenous injection into a peripheral vein. For 24 hours after administration of Foscan® the patient remained in a darkened room (curtains drawn, light bulbs not brighter than 60W). The patients made a gradual return to unrestricted light exposure over two weeks. Permissible light levels were 100 Lux on the day of injection, increasing by 100 Lux per day. Guidelines for light exposure and a

light meter were given to patients prior to the initiation of treatment, and investigators were advised to tell the patients and their families or friends who attended them during the treatment about the importance of avoiding sunlight. Patients were to avoid high levels of ambient light for a month and avoid ophthalmic procedures involving fundoscopy for three months after administration of Foscan®.

The total light dose for treatment was 20J/cm² at a light intensity of 100mW/cm². Treatment times were 200 seconds. The illuminated area extended beyond all visible tumour margins by a distance of at least 0.5cm. This is in keeping with the surgical approach to the management of these tumours where a margin of normal tissue is included in the resection.

9.4.5 Overall Results (combined from all centres)

The complete response rate in the patients treated according to protocol was 85% (97/114) at twelve weeks. Response rates were higher for smaller tumours (Table 9.6). Of the 17 patients not documented as having a complete response at 12 weeks, 1 was not assessed, 1 refused further treatment and died of extensive disease one year later. 2 patients were initially lost to follow up but were subsequently traced and found to be disease free. The remaining 13 were partial responders who went on to receive other therapy. Of these, 9 obtained a CR. 5 had disease progression and have since died (Table 9.7).

T_{is}	T₁	T₂	Overall	Confidence intervals
3/3 (100%)	83/92 (90%)	11/19 (58%)	97/114 (85%)	77 - 91

Table 9.6: Complete response rates by tumour T-stage

Partial responders, withdrawals and patients lost to follow up N = 17/114; CR = 10/17; PR = 7/17	
1	Not able to assess; died nine weeks after treatment of intercurrent illness
1	Patient lost to follow up (refused treatment)but found one year later with extensive disease which was too advanced to treat. Patient died of progressive illness
2	Patients initially lost to follow up – subsequently traced and found alive and disease free
1	PR, treated again with PDT: CR
6	PR, treated with surgery: four CRs, two died of progressive disease
2	PR, treated with radiotherapy: one CR, one died of progressive disease
3	PR, treated with surgery and radiotherapy: two CRs, one with advanced disease
1	PR, treated with radiotherapy and chemotherapy: died of advanced disease, not clear if new tumour or recurrence

Table 9.7: Partial responders, withdrawals and patients lost to follow up.

Key: CR-complete response; PR-partial response

The 12 week post-treatment biopsy was undertaken for purposes of confirmation of efficacy. However, where the treated areas were assessed clinically as being normal, no biopsy produced evidence of residual tumour.

9.4.6 Toxicity and Side Effects

In contrast to treatment with either surgery or radiotherapy, Foscan® PDT was relatively well tolerated and free of major toxicity. There were a number of transient alterations in haematology and biochemistry, but no overall trend was identified as being of clinical significance.

Adverse events in the immediate post-treatment phase included pain and swelling in most patients requiring short-term treatment with opiates in addition to non-steroidal anti-inflammatories. Pain symptoms commonly persisted for between two and four weeks. Although some patients experienced little post-treatment pain, others required moderate analgesic support for up to twelve weeks. At the treated site, there was localised pain and tissue necrosis: there appears to be a clear correlation between reported pain and leukocytosis (Figure 9.3). This probably reflects the necrosis and separation of the necrotic

tissue, which is the cause of the leucocytosis. In addition, there was oedema and an inflammatory response that usually resolved within three weeks.

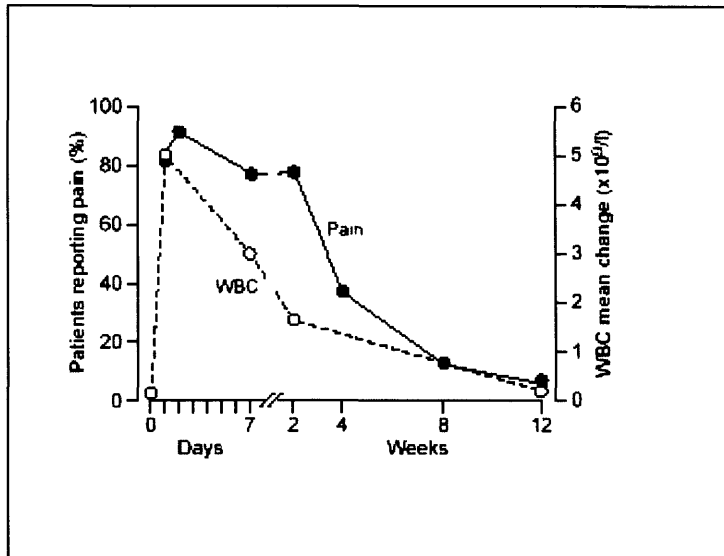


Fig 9.3: Pain and leukocytosis following treatment with Foscan® PDT

Photosensitisation did not appear to be a significant problem. 16 patients (13%) exhibited photosensitivity reactions: in 14, this was only mild erythema with no long-term sequelae. Only 2 of these were considered to be significant in patients who failed to comply with the light regimen. One of these required skin grafting to a burn on his arm. The low incidence of light-related complications was due to adherence with the light exposure regimen described above and the value of careful pre-treatment counselling cannot be over-emphasised. Scarring at the treatment site was noted in 11 patients, but only severe in 1.

9.4.7 Survival

1 patient died during the initial 12 week follow up period of the study: the death was not considered to be related to treatment with Foscan® PDT. 10 further deaths occurred within the first year: 2 from unknown causes, 2 from metastatic disease, 2 from progressive disease and 4 from unrelated causes. In the second year, there were 11 further deaths: 3 from metastatic disease, 4 from progressive disease, 1 unknown and 4 from unrelated causes. The four patients who died of metastatic disease in the first 2 years of the study all showed complete response at the primary site.

The complete response rate at one year was 86% and at two years was 79% (Figure 9.4).

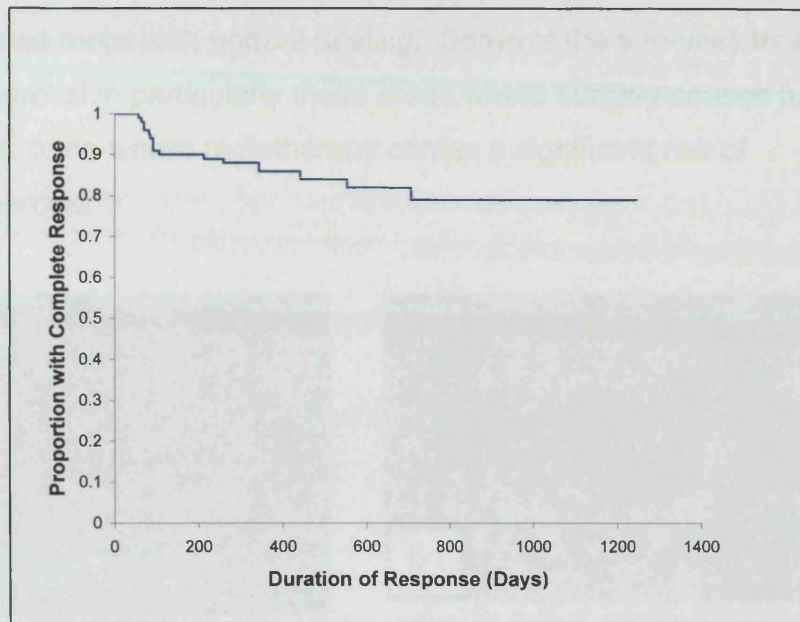


Fig 9.4: Duration of complete response, $n = 95$

Mean duration of complete response = 621 days (se=24)

1 year = 86%, 2 years = 79%

The survival rate at one year and two years was 90% and 77% respectively (Figure 9.5).

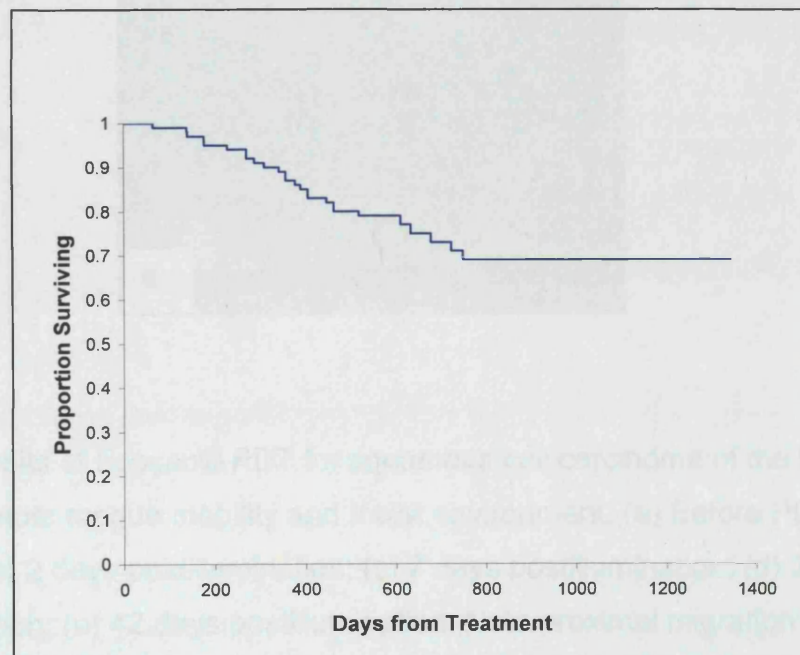


Fig 9.5: Survival

$n = 113$; Mean 1 year survival rate = 90%, 2 year survival rate = 77%

All successfully treated patients had ablation of their disease without significant tissue loss or reduction of function in terms of speech and swallowing. The mouth remained moist with normal healing. Some of the sub-sites treated were especially impressive particularly those areas where surgery causes functional deficit, or over bone where radiotherapy carries a significant risk of osteoradionecrosis.

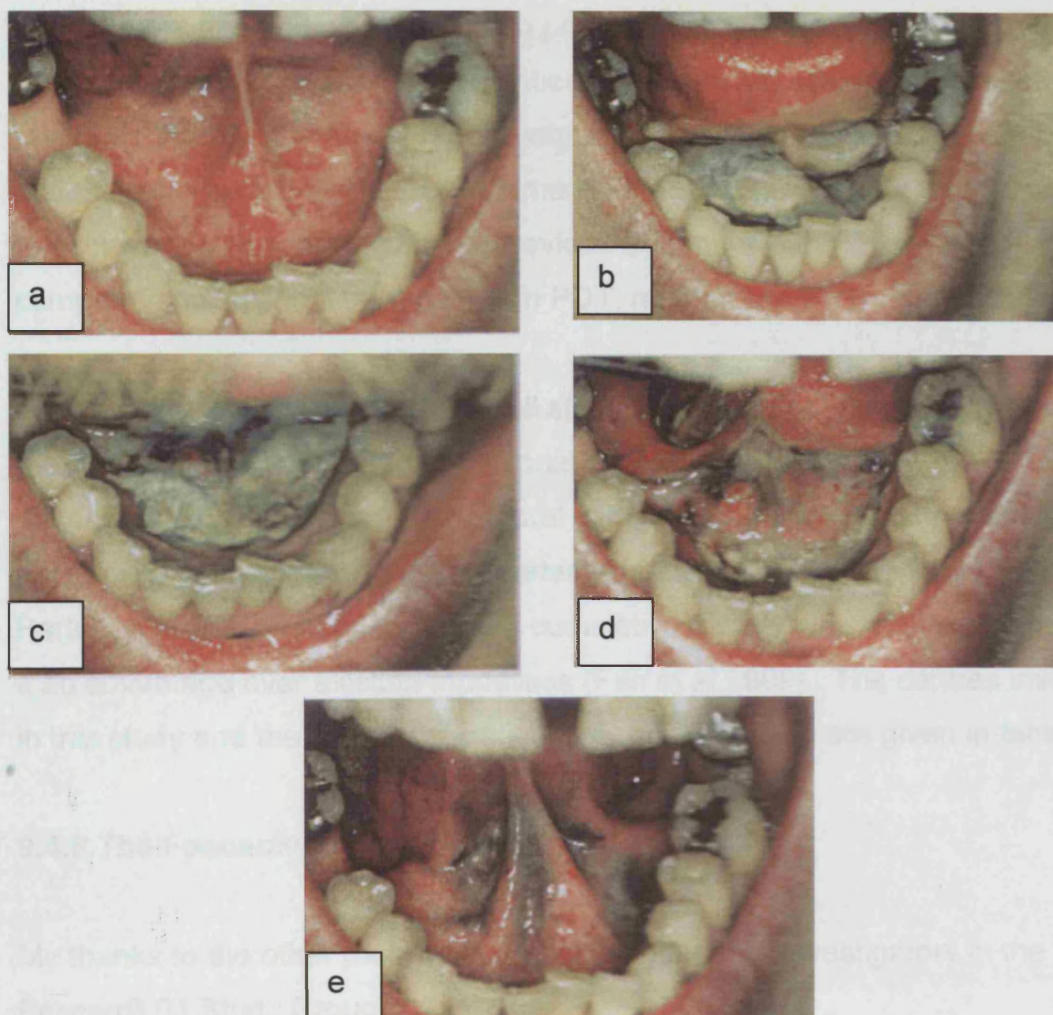


Fig 9.6: Results of Foscan® PDT for squamous cell carcinoma of the floor of the mouth - note tongue mobility and moist environment. (a) Before PDT treatment; (b) 2 days postillumination; (c) 7 days postillumination; (d) 21 days postillumination; (e) 42 days postillumination. Note proximal migration of submandibular duct orifice without glandular compromise. (Courtesy A Kübler, Cologne)

The response rates seen with Foscan® PDT compare favourably with response rates seen with surgery and radiotherapy (Fein et al 1994, Laramore 1989, Wolf et al 1993, Shah et al 1999). A direct comparison is impossible as most other studies collected data retrospectively and not on an intention to treat basis. Not only that, the other large studies comprised a heterogeneous mix of tumours. Histological confirmation of complete response was obtained in 71% (biopsy was refused by the remaining 29%). In those patients who had a complete clinical response and who also had a post-treatment biopsy, not a single clinical false negative was found. Those patients who did not achieve a complete response still had the option of re-treatment or conventional therapy. Of the 15 patients who went on to further treatments it is important to note that 8 went on to achieve a complete response. Previous treatment with Foscan® PDT did not compromise additional treatment with PDT, radiotherapy or surgery if indicated.

Long term follow up data from the full study are limited at this stage, however the one- and two-year complete response rates of 86% and 79% are encouraging. The genetic basis of oral cancer evolution suggests that these patients would have a high risk of metachronous tumours (De Vries 1990, Partridge et al 1997) and the lack of cumulative toxicity with this treatment gives it an advantage over existing modalities (Fan et al 1999). The centres involved in this study and the number of patients recruited at each are given in table 9.8

9.4.8 The Foscan® 01 Study Group

My thanks to the other participating study centres and investigators in the Foscan® 01 Study Group:

France: T Patrice, C Beauvillain, Clinique ORL, Nantes. **Norway:** J Evensen, Norwegian Radium Hospital, Oslo. **South Africa:** K Butow, Department of Maxillofacial Surgery, University of Pretoria; B Smit, Department of Radiation Oncology, Tygerberg Hospital, Tygerberg. **United Kingdom:** J Brown, Fazakerly Hospital, Liverpool; J de Carpentier, Royal Preston Hospital, Preston; J Carruth, Southampton General Hospital, Southampton; M Dilkes, G Kenyon, St Bartholomew's Hospital, London, F Roberts, Cookridge Hospital, West Yorkshire; N Sudderick, Royal Surrey Hospital, Guildford, Surrey

Study identification	Institution	Patients entered
101 - 105	Royal London Hospital UK	5
201 – 213	University College London Hospitals UK	12
301 - 302	Southampton General Hospital UK	2
401 – 407	National Cancer Institute Amsterdam The Netherlands	16
501 – 504	Royal Surrey Hospital UK	4
600 – 623	University Hospital Köln Germany	24
701 + 706	Norwegian Radium Institute Oslo Norway	2
801 – 824	The Ulster Hospital Belfast UK	22
901	Clinique ORL Nantes France	1
1001 - 1004	Tygerberg Hospital South Africa	4
1101 + 1102	Cookridge Hospital UK	2
1201 – 1205	Fazakerley Hospital Liverpool UK	5
1401 – 1411	Cumberland Infirmary Carlisle UK	10
1501 – 1510	Royal Preston Hospital UK	9
1601 – 1603	University of Pretoria South Africa	3
	Total enroled	121

Table 9.8 Recruitment: total of 121 patients, 15 centres, 5 countries. 7 patients were withdrawn at an early stage due to protocol violations as indicated in table 9.4.

9.4.8.1 UCLH Patients

The 12 patients UCLH patients are included in the full analysis given above, but for completeness, the results for these individuals are summarised here. All except 1 were T1 tumours with a mean maximum diameter of 1.3 cm and depth of 2.8mm compared with a mean diameter of 1.7mm and mean depth of 3mm in the main study. Their mean age was 59 years with a slight female preponderance (Table 9.9). This is similar to the total study group. Out of the 12, only 1 had a metachronous tumour requiring treatment within the 2 year follow up. One died of a perforated duodenal ulcer and this might well have been secondary to post treatment analgesic use.

Patient	Stage	Depth (mm)	Site	Age (years)	Sex
1	T1 (1.3cm)	3	P	68	F
2	T1 (1.8cm)	4	MA	46	M
3	T1 (1.5cm)	5	FOM	42	M
4	T1 (1.0cm)	2	T	49	F
5	T1 (1.7cm)	3	T	78	F
6	T2 (2.3cm)	4	FOM	54	F
7	T1 (0.8cm)	1	T	56	F
8	T1 (1.0cm)	2	MaxA	62	F
9	T1 (1.7cm)	4	FOM	48	M
10	T1 (0.5cm)	2	T	78	M
11	T1 (1.4cm)	2	BM	63	M
12	T1 (1.8cm)	1	P	68	F
Mean	1.3cm	2.8mm		59 (42-78) years	7F 5M

Table 9.9: Patients recruited to the 01 Study from UCLH

P: Palate **M:** Mandible **A:** Alveolus **FOM:** Floor of Mouth **T:** Tongue

Max: Maxilla **BM:** Buccal mucosa

The longer term follow up was a little less impressive as demonstrated by the 5-year follow up on these 12 patients. Nearly half of this group are dead (5/12) although only one died of disease progression. 2 died of an unrelated cause, 2 died from metachronous tumours and of the remaining group that are alive, 2

have been successfully treated for metachronous tumours (Table 9.10). This is hardly surprising given the high levels of comorbidity associated with oral cancer patients, but reinforces the need for effective treatments with reduced long term morbidity.

Patient	Foscan® date	Histological CR	Progress	Outcome	Comments
1	03.05.96	28.08.96	Withdrawn 22.10.96	Died 723 days	Metachronous tumour S R C
2	08.08.96	07.11.96		AW 7.3y	
3	18.10.96	26.02.97	No primary site disease	AW 7.5 yrs	Metachronous Palate S Pharynx R
4	07.12.96	30.04.97	Withdrawn 22.10.98 S	Died 29.05.01(4.5y) Cardiomyopathy	Metachronous tumour S
5	03.02.97	02.04.97	No primary site disease	Died 18.12.03 (6.8y)	Multiple metachronous tumours PDT
6	28.08.97	16.09.97	No primary site disease	Died neck disease 16.09.99 (749d)	
7	12.10.97	06.01.98	No disease	AW 6.6y	
8	02.04.98	02.06.98	No disease	AW 6y	
9	22.05.98	30.06.98	No disease at primary site	AW 6y	Metachronous tumour
10	13.08.98	AE Death	Clinical CR	Died 139d	Perf DU/renal failure
11	09.11.98	Withdrew		AW 5.5y	Tracked to Ireland – recent check no disease
12	25.02.99	15.06.99	No disease	AW 5.5y	

Table 9.10: Results from study patients with T₁ disease treated at UCLH with Foscan® PDT

Key:- S - Surgery R – Radiotherapy C - Chemotherapy

These data support the safety of Foscan® PDT in the treatment of early oral SCC. The perceived problems of prolonged photosensitivity have not been observed in this study as residual photosensitivity of the drug is low compared with first generation sensitisers. Patients were able to monitor incremental light exposure in the post-sensitisation period. Given the age and socio-economic status of the oral cancer population, it is notable that the level of photosensitivity complications remained low. This was related to the strictly increasing light regime that patients followed, and the self-monitoring that was carried out using portable light meters.

9.5 Discussion

The management of the N₀ neck in early oral cancer remains controversial (Yuen et al 1997, Leemans et al 1998, Pitman et al 1997, Gallo et al 1996). In most treatment protocols the neck is managed with either an elective neck dissection or close observation. In this study the close observation protocol was utilised. During the recorded period of follow-up, three patients at UCLH developed neck metastases, which were managed with conventional treatment.

Pain at the treatment site was managed with a combination of opiate, opioid and NSAID analgesia. Pre-emptive analgesia was found to be the most effective way of managing post-treatment pain. Analgesia was found to be necessary in most patients for up to four weeks.

Infection was rarely found to be a problem. In the moist environment of the oral cavity, necrotic slough was sometimes associated with a fetor, which responded to the use of topical or systemic metronidazole. In general, treated sites re-epithelialised within six weeks. In certain sensitive sites such as the soft palate or posterior tongue, the use of dexamethasone to reduce post-treatment oedema was found to be useful.

Treatment of tumours overlying bony structures may result in the exposure of bone. Even when this occurred, spontaneous resolution took place, although re-epithelialisation was delayed. Throughout this study no patients required any form of airway management or enteral feeding. No patients required nutritional

support apart from minor dietary modifications in the immediate post-treatment phase.

Normal laser safety precautions were required for the patient and the operators. In addition to the general light precautions outlined above it is important to shield surrounding normal tissue from reflected light during treatment. This is most commonly achieved by the use of surgical drapes, gauze and copper wax.

It is possible to carry out many treatments using local anaesthesia, with or without intravenous sedation. As some of the PDT effect is thought to be directed at the vasculature, adrenaline free anaesthetics were used for infiltration although we consider it preferable to use lidocaine with adrenaline for regional blocks. General anaesthesia is often given to facilitate access to the more difficult sites within the oral cavity. Light exposure in many patients is in fact painless.

The short light activation time of 200 seconds is due to the potency of Foscan® and confers an additional treatment benefit which enables some treatments to be carried out on an outpatient basis. The equipment costs for this treatment are modest. Although the drug cost is high, we believe that Foscan® PDT will offer a cost-effective alternative to radiotherapy or surgery (Hopper et al 2004a).

9.6 Comparison Between the Three Photosensitisers

There is a paucity of prospectively acquired information on treatments for head and neck cancer generally. The decision making process for management of patients with head and neck cancer is largely based on retrospectively acquired evidence which often consists of a heterogeneous mixture of clinical stages and anatomical sites of disease.

9.6.1 Photofrin®

A small number of patients were treated using this drug. Only 4 pureT1 patients were treated. In 1993, there were compelling reasons for abandoning the use of this drug. Treatment times were long – typically 1000s, the light sources

available at the time were metal vapour pumped dye lasers that were unreliable and required high maintenance and the long term photosensitivity (up to 3 months with Photofrin®) was a nuisance. Outcomes were very good both in terms of the cosmetic and functional results with 100% cure rate at mean follow up of 4 years. This is despite the fact that treatment depth was limited due to the moderate tissue penetration of the activation wavelength (Grant et al 1997).

Since then, although many of the original problems persist, the laser light can now be generated by diode lasers that are portable, reliable and relatively maintenance free, so Photofrin® might still have a role to play in the management of early oral cancer. From the histological studies in Chapter 7, there really is good preservation of collagen and elastin which is superior to Foscan® and if a moderate depth of effect is required and prolonged phototoxicity is not an important issue, Photofrin® again might still have a place in the management of early oral cancer.

9.6.2 ALA

Some benefit was observed in five of six patients with squamous cell carcinoma, but only two became tumour free (one with persistent mild dysplasia). Clearly, it is inappropriate to continue with this approach to invasive SCC. It might be possible to increase the depth of effect of ALA PDT by changing treatment parameters. There has been an ongoing debate as to the optimum wavelength of light for maximum depth of treatment effect and it seems that 635nm probably gives an effect twice as deep as 628nm. Treatment breaks and the addition of iron chelators might be of value, however, the effect is so superficial that it seems unlikely that ALA will have a major role to play in the management of oral cancer.

9.6.3 Foscan®

This is the largest prospective study of PDT treatment of early oral cancer to date (there has been one larger study on advanced cancer treated with Foscan® PDT that includes 128 patients - D'Cruz et al 2004). Foscan® PDT was shown in this study to be a safe effective way of treating early oral SCC.

There is the potential for some scar formation but there are still clear advantages over conventional treatment in terms of improved function and cosmesis and PDT should be available to all integrated head and neck oncology teams.

9.7 Summary

ALA PDT has too superficial an effect to be of real value in treating invasive oral cancer, however, both Photofrin® and Foscan® are very effective and probably as good as radiotherapy and surgery as a primary treatment. The limiting factor in making this comparison is not the quality of data for treatment outcomes with PDT rather the complete absence of outcome data from conventional treatment. The above (Foscan®) study is the only intention to treat study in the literature of early oral cancer, which is a damning indictment of research into head and neck cancer.

Chapter 10: A Study to Investigate the Efficacy of Photodynamic Therapy in the Treatment of 'Field Cancerisation' of the Oral Cavity

10.1 Introduction

Section 2.2 described the greatly increased chance of developing a second primary cancer when pre-malignant lesions such as leukoplakia and erythroplakia co-exist with an existing primary (Sarasin 1933, Wilkins and Vogler 1957, Moertal and Foss 1958, Shibuya et al 1986). Leukoplakia, a term describing a white patch or plaque that cannot be characterised clinically or pathologically as any other disease entity (Kramer et al 1978) has a definite propensity for malignant transformation. This is variously estimated as being between 0.13 and 17.5 per cent (Pindborg et al 1968, Silverman et al 1976, Silverman et al 1984). The presence of an erythematous component, erythroplakia, or erythroleukoplakia increases almost four-fold the risk of malignant transformation (Silverman et al 1984).

The incidence of multiple oral carcinomas in patients with primary tongue carcinomas associated with leukoplakia has been found to be five times greater than that of subjects without leukoplakia (Shibuya et al 1986). The prognosis of patients who develop synchronous or metachronous second primary cancers is poor with overall five-year survival ranging from nine to 22 per cent (Marchetta et al 1965, Gluckman and Crissman 1983). Slaughter et al (1953) first proposed the concept of field cancerisation to explain the multi-centric origin of squamous cell carcinoma arising in a given anatomical region, exposed to the same carcinogenic agents. The diagnosis of a secondary primary tumour in the same anatomical site, or the co-existence of pre-malignant lesions such as leukoplakia or erythroplakia, suggests that the entire mucosa is at risk of malignant change. Satisfactory treatment for patients with such a wide field change should ideally be non-mutilating and repeatable.

Early clinical experience with PDT showed that it was effective in the treatment of superficial lesions, particularly those such as urothelial bladder cancer, early bronchogenic carcinomas and dermatological tumours (Hayata et al 1984, Benson 1988, Carruth 1990, Edell and Cortese 1990, Pope and Bown 1991a).

Variable outcomes have been reported in the treatment of head and neck cancers. Possible reasons for failure include inappropriate patient selection, or failure to expose deep extensions of the tumour to adequate light (Gluckman 1991a).

The patient with wide field change in the oral cavity presents a difficult management problem. Screening of these patients results in detection of areas of invasion while still at an early stage. The ability of PDT to ablate superficial tumours, while at the same time minimising loss of normal tissue, suggests that it should be ideally suited to the treatment of this disease.

10.2 Photofrin®

The first study reports clinical experience with photodynamic therapy using Photofrin® Lederle Laboratories (at the time of the study) in the treatment of superficial early carcinomas with co-existing premalignant lesions and areas of carcinoma in situ occurring in the oral cavity. Photofrin® PDT has been shown to be capable of destruction of tumour to an approximate depth of 0.5cms and still heal with little or no scarring. It would therefore seem to be a reasonable technique to use in the management of field change disease. Clearly it is better in the treatment of superficial disease and not really appropriate for thicker tumours, although the extent or number of lesion is not a limiting factor. The first study was carried out to investigate the potential of Photofrin® PDT for the treatment of field change disease.

10.2.1 Materials and Methods

Eleven patients with one or more histologically proven early invasive squamous cell carcinomas of the oral cavity, occurring in association with widespread premalignant disease such as leukoplakia, erythroplakia or 'erythroleukoplakia' were included in the study group. Six patients had synchronous or metachronous second primary carcinomas, and five had a first primary lesion, occurring in association with one or more non-contiguous areas of leukoplakia of more than 5mm in diameter (see Table 10.1).

Pt	Tumour site	Associated pre-malignant disease	Appearance of treated areas at 6-8 weeks	FU (mths)	Comments
Primary cancer with associated pre-malignant disease					
1	R bucco- gingival sulcus	R and L buccal mucosa	CR, Normal*	10	HIV positive
2	R lateral tongue	R tongue base, L buccal mucosa	CR, Normal	8	Leukoplakia at 6 months; mild dysplasia
3	L buccal mucosa	L buccal mucosa – 3 sites	CR, non- healing ulcer	9	Ulcer excised at 3 months: inflammatory
4	Tongue (excised)	L and R buccal mucosa	CR, Normal	3	
5	L bucco- gingival sulcus	L buccal mucosa	CR, Persistence of OSF	19	Erythroplasia 1 year – moderate dysplasia. OSF
Multiple primary with associated pre-malignant disease					
6	L buccal mucosa – 2 sites	L buccal mucosa – 2 sites	CR, Normal	9	
7	R alveolar ridge – 2 sites	R buccal mucosa, floor of mouth	CR, Normal*	10	Metachronous primary 8 months
8	L alveolar ridge and buccal mucosa	R buccal mucosa and alveolus	CR, Normal	7	
9	Tongue tip L lateral tongue	Dorsum and R lateral tongue	IR, Some leukoplakia	18	Biopsied; moderate dysplasia
10	Palate/L buccal/R alveolus	R tongue and buccal mucosa	CR, Normal*	6	Metachronous primary 3 months
11	Lip - 2 sites	Lip	CR, Normal	18	

Table 10.1: Photofrin® PDT in field change disease

Key: **Pt**-patient; **FU**-follow-up; **CR**-complete response: no evidence of abnormal mucosa, either histologically or clinically, at time of assessment; **IR**-incomplete response: residual pre-malignant lesion in treated areas; **OSF**-oral submucous fibrosis; *-biopsied: histologically normal mucosa.

All tumours were diagnosed as Stage 1 (T₁N₀N₀) oral cancers and no deep extension to underlying muscle or bone was suggested by the clinical, histological or radiological findings. Six patients had had previous oral tumours surgically excised, five on more than one occasion, and now presented with independent second primary carcinomas. One patient had previous CO₂ laser excision of dysplastic areas of leukoplakia. Four patients had no prior treatment but had histologically proven squamous cell carcinomas associated with one or more non-contiguous areas of leukoplakia. One patient underwent a combination of surgical resection of a T1 lesion of the tongue and PDT to extensive areas of leukoplakia in both buccal regions. No patient had received radiotherapy. Informed consent was obtained in each case.

Photofrin® at a dose of 2mg/kg was given by intravenous infusion 48 hours prior to surface illumination of the tumour and all clinically apparent areas of leukoplakia or erythroplakia. A copper vapour pumped dye laser was used to deliver monochromatic red light at 630nm. An optical fibre with a microlens affixed ensured homogeneity of the illuminating light spot, and a multi-jointed articulated arm was used to facilitate positioning of the fibre in three planes.

Two patients were treated for very extensive disease on two separate occasions. Ten patients required more than one spot to treat the target areas. A light dose of 50 to 100J/cm² was delivered to each area. Power densities were kept below 150mW/cm² to avoid any possibility of thermal injury, and no patient reported any sensation of heat, although some described various sensations such as tingling and tickling during light exposure. Patients were counselled to avoid direct sunlight following sensitisation. Patients were prescribed analgesics and benzydamine local analgesic or anti-inflammatory spray.

Lesions were photographed and treatment areas were recorded diagrammatically on referral. Following treatment patients were reviewed and photographed twice in the week after PDT and then approximately weekly until healed, and thereafter every two to four weeks.

Where treated areas healed with, or subsequently developed, evidence of recurrence of ulceration, erythroplasia or leukoplakia, these lesions were conventionally excised or biopsied to determine the nature of the abnormality and the presence or otherwise of pre-malignant or malignant recurrence.

Three patients had biopsies of regenerated clinically normal mucosa, at sites where invasive carcinoma had been previously diagnosed. Each was taken more than three months after PDT.

10.2.2 Results

All treatments were performed without local anaesthetic or sedation and were satisfactorily tolerated by each patient, on an out-patient basis. During the three to four days after PDT the treated areas underwent obvious surface necrosis with the development of a superficial slough, and local oedema. While no pain was associated with the treatment itself, all patients reported some degree of pain over the following days, usually lasting about one week, but this was effectively relieved by oral analgesia. Eating tended to exacerbate pain and a sloppy diet was advised and found to help. Treated areas took between three and five weeks to heal fully, depending on the size of area treated.

At assessment following healing, six to eight weeks after PDT, 10 of the 11 patients showed a complete response to treatment. In eight, the healed mucosa was of normal colour with no evidence of leukoplakia or erythroplakia (Figures 10.1 a and b).

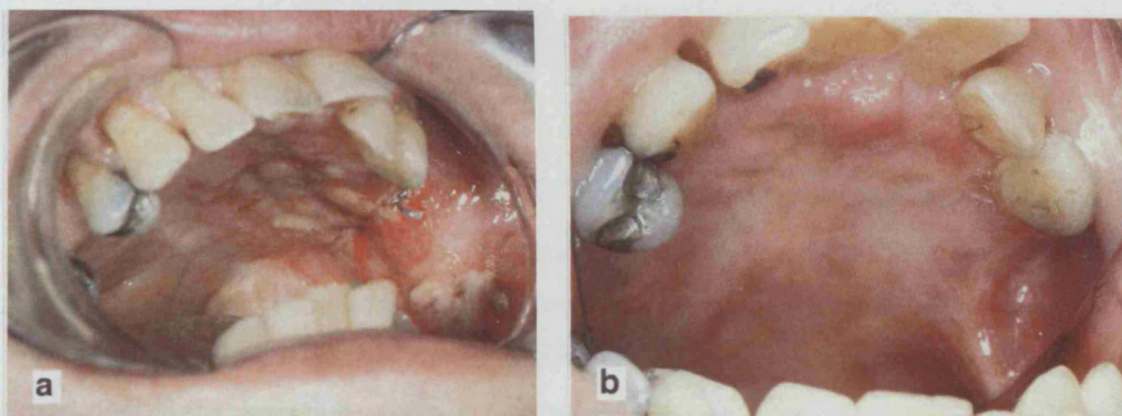


Fig 10.1: Patient 10: before (a) showing invasive squamous cell carcinoma of the left hard palate and buccal mucosa; and (b) three months after Photofrin® PDT showing appearance of palate following healing

Five patients who had had multiple surgical resections (patients six to ten, Table 10.1) showed evidence of scarring related to previous surgical intervention, but healed with essentially normal mucosa. The three cases in which clinically normal regenerated mucosa was biopsied after three months, showed histologically normal epithelium in two cases (Figures 10.2 a and b) and an atrophic epithelium without atypia or dysplasia in the third.

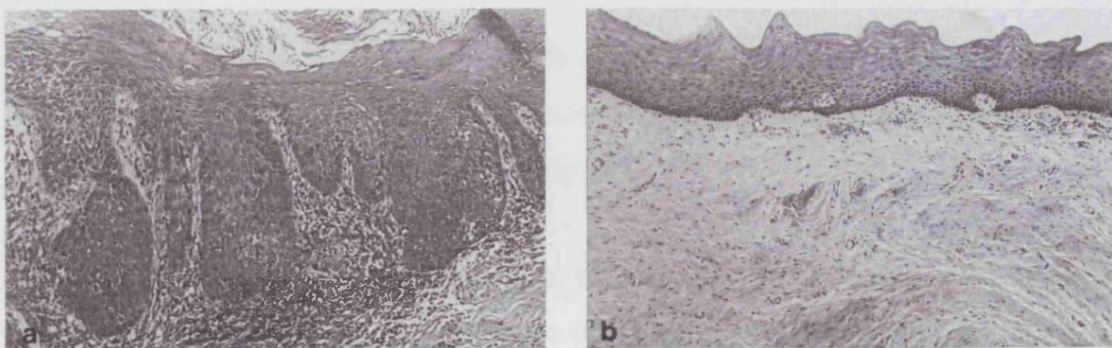


Fig 10.2: Photomicrographs of histological sections showing: (a) early invasive carcinoma of the right lower bucco-gingival sulcus before Photofrin® PDT and (b) showing appearance following healing, with normal mucosa.

One patient (Pt 5) with pre-existing oral submucous fibrosis healed with scarring, similar in appearance to adjacent untreated mucosa. One patient (Pt 3) had a persistent non-healing ulcer associated with dental trauma. This was

excised after three months and showed simple ulceration with chronic inflammation.

One patient (Pt 2) had an incomplete response, but had almost the entire surface of his tongue treated in two separate sessions. More than 75% healed with regeneration of mucosa of normal colour but with some atrophy. The persistence of a patchy grey-white lesion on the dorsum, showing histological mild dysplasia, meant that this constituted an incomplete response (Figure 10.3 a and b). However, the patient has undergone several re-treatments and is currently alive and well ten years after this study. Interestingly he has remained disease-free over the last three years.

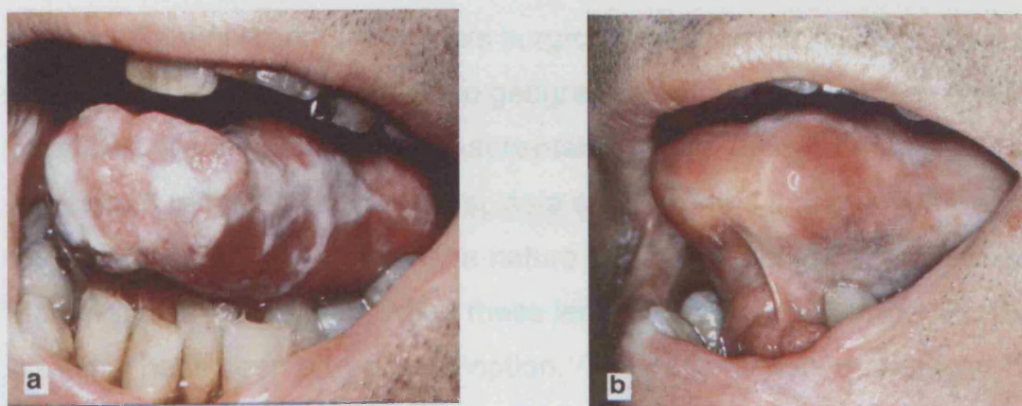


Fig 10.3: Patient 9: before (a) and after (b) PDT for extensive field cancerisation of the tongue. Patient had multiple prior surgical resections of tongue and lower lip. Appearance at 16 months following PDT

Follow up ranges from 3 to 19 months, with a mean of 11 months. No patient developed any evidence of recurrence of invasive squamous cell carcinoma in the treated sites. One patient developed a small area of leukoplakia at the treatment site at six months. This was excised and histology showed hyperkeratosis with moderate epithelial dysplasia.

The patient with oral submucous fibrosis developed further erythroplakia at one year which, when excised, also revealed moderate epithelial dysplasia. Two patients developed further metachronous primary carcinomas at sites away from the treated areas, while maintaining a complete response in the treated area. These were treated with excision and radiotherapy.

The remaining seven patients had no evidence of recurrent carcinoma or further pre-malignant disease.

10.2.3 Discussion

The complete response of 10 of the 11 patients treated in this study demonstrates the efficacy of Photofrin® PDT in ablation of early invasive and pre-malignant lesions in the oral cavity. The finding that in most cases the abnormal epithelium healed with regeneration of essentially normal mucosa indicates that early superficial carcinomas and associated dysplastic lesions can be effectively destroyed in situ with minimal tissue loss using PDT.

For simultaneous primary cancers surgical resection may be feasible if two or more primary lesions are in close geographical proximity, but may require extensive reconstruction with unacceptable loss of function. The two cases that developed recurrent areas of dysplasia at six months and one year respectively were disappointing but, given the nature of their field change, not unexpected. Both patients subsequently had these lesions excised, but further PDT would certainly have been a treatment option. The development of metachronous primary cancers in two patients is also not unexpected and again reflects the field change nature of the disease rather than any influence of PDT, as the sites were not related.

The incidence of second or multiple cancers arising in the upper aerodigestive tract has a profound influence on survival, and therefore management (section 2.2). Management of areas of dysplastic oral mucosa is a considerable problem because of multiple sites, often diffuse margins and variable and unpredictable risk of malignant transformation. When leukoplakia occurs in conjunction with one or more primary tumours, indicating the likelihood of a wide field change, these difficulties are greatly compounded.

Therapeutic options for patients with extensive field cancerisation will depend on several factors, including the timing of presentation of the tumours, whether simultaneous or metachronous, the site and stage of the lesions, and the age and general health of individual patients. Concern has frequently been

expressed about the possibility of radiotherapy converting pre-malignant disease into anaplastic and frankly invasive carcinoma, and the long-term effect of radiation (section 2.2.2). Robinson et al (1991) report decreased mean survival from 102 months to 55 months for second primaries compared with first and this fell to 20 months if the first primary had been irradiated. For metachronous lesions curative surgery is undertaken on the merits of the individual case, but the problems of function and cosmesis are the same.

Photodynamic therapy has been shown to be effective in vitro and in vivo for the ablation of small areas of malignant disease. Although current photosensitisers do not have a totally selective effect, the ability of surrounding normal tissues to heal by regeneration results in ultimate selectivity of tumour destruction (see section 4.1.4 and Chapters 5 and 6).

Clinical studies demonstrate excellent healing of skin and mucosal surfaces following PDT with Photofrin® (Gilson et al 1988, Barr et al 1990).

Photodynamic therapy results in minimal tissue loss with preservation of function. This study demonstrated that diffuse areas of superficial malignancy and pre-malignant disease can heal with clinically or histologically normal mucosa following treatment.

Early clinical reports of PDT in the head and neck comprise feasibility studies in patients with advanced disease and demonstrate variable tumour response and palliation. Visible light penetration is maximal in the red region of the spectrum, but is not capable of causing necrosis more than a few millimetres from the surface so that surface illumination of bulky tumours is likely to be of little value.

More recently it has been suggested that PDT is more appropriate for the treatment of superficial lesions. Wenig et al (1990) used PDT to treat 26 patients with early stage head and neck cancer who had refused conventional treatment or had failed previous treatment of surgery, radiotherapy, chemotherapy or combination therapy. These authors used an idealised mathematical light dosimetry model to match light delivery to magnetic resonance imaging of tumour volume.

Complete responses were reported in 20 (77%) and partial responses in five (19%) as assessed at three months. Nine of these patients had oral cavity tumours. Unfortunately there appear to be a wide variation in the optical properties of individual tumours and few conform to idealised geometries (Grant et al 1992).

Gluckman (1991b) treated 13 early (T_1 and T_2) carcinomas of the oral cavity and oropharynx by surface irradiation. He obtained complete response in 11 (84%) at one month, with the remaining two being partial responses. However, of these 11, four recurred between eight and 12 months. He concedes that this high late recurrence rate may have been due to a variety of factors and expresses concern about the possibility of deep tumour being left untreated.

The same author, in the only other study to address field cancerisation patients, reports results in eight patients with condemned mucosa (defined as biopsy proven multi-centric pre-malignant and overtly malignant lesions). He obtained excellent results in seven patients in the short term. A number developed recurrent leukoplakia on long-term follow up but only one was positive on biopsy. Seven out of eight patients with condemned mucosa had a complete response at follow-up from 6 to 53 months.

He found that results achieved in aiming to palliate eight patients with advanced cancer were disappointing and in fact skin photosensitivity may have worsened their quality of life and therefore he abandoned PDT for this indication. These and other experiences with PDT both in the head and neck and in other situations have contributed to the growing body of opinion that PDT should be confined to the treatment of early superficial malignancies. However, improved photosensitisers and interstitial techniques have made the treatment of large tumours a viable option.

10.2.4 Conclusions

If histological examination confirms that the lesion is confined to the mucosa then PDT with Photofrin® is an effective management option. However, Photofrin® in this setting is less than ideal due to the failure of light at the

activating wavelength to penetrate more than a few millimetres beneath the surface (section 4.1.3). Also, the prolonged residual photosensitivity can be difficult for patients to accept.

10.3 ALA PDT

At the outset of clinical investigation of ALA there was great optimism about the PDT effect. It was thought possible that this technique could be used to treat cancer down to a similar depth as Photofrin®. Initial studies suggested that PPIX accumulated in malignant tissue, but was virtually absent from normal tissues other than epithelium. The light of 635nm had been shown to penetrate 0.5cm so it was assumed that the effect could be generated to this depth. This, however, was not the case – the PDT effect was only to a depth of about 1mm, so not surprisingly, ALA PDT was soon abandoned for this indication, however, a small number of patients were treated.

10.3.1 Materials and Methods

Three patients with histologically proven field change disease of the mouth were sensitised with 60mg/kg ALA by mouth and treated with laser light at 628nm (100 or 200J /cm²). The results were assessed macroscopically and although microscopic assessment was also planned, this was only possible in one patient. The study was approved by the hospital ethical committee, and all treatment was carried out with fully informed consent from the patients.

ALA was supplied for this project as the hydrochloride in powder form by DUSA Pharmaceuticals (Tarrytown, New York). For clinical use, this was dissolved in orange juice and given by mouth in three separate doses of 20mg/kg at 0, 1, and 2 hours (total dose, 60mg/kg) using the regimen established by Regula et al (1995). The light source used was a gold vapour laser giving red light at 628nm (Dynamic Light, Milton Keynes, UK). This was delivered to the patient using a single mode scrambled, 0.4mm flexible optical fibre with a bare flat-cleaved tip that gave a circular spot of light up to 2.5cm in diameter on the target tissue. The area to be treated was defined as the target lesion together with a margin of 5mm of surrounding normal tissue. Appropriate fibre positions

and treatment times were calculated to give the desired light doses before treatment started, using one of the two dose regimens described in Chapter 7. One patient was treated with $100\text{J}/\text{cm}^2$ and the other 2 were treated using the $200\text{J}/\text{cm}^2$ interrupted treatment regime. If possible, the entire lesion was treated from a single fibre position. The power density was kept below $250\text{mW}/\text{cm}^2$ to avoid thermal effects. As many of the total exposure times were quite long (up to 143 minutes), the fibre was positioned in a multi-jointed arm to keep its tip in the correct place over the area to be treated. Patients were given systemic analgesia and topical anaesthetic with occasional sedation if necessary. Injected local anaesthetic was limited to the site of biopsy.

A diagnostic biopsy specimen was taken before patients were considered for inclusion in this study, further biopsies were performed when healing was complete and at long term follow-up to assess treatment results.

Patients were kept in a dark room for 24 hours after receiving ALA. Routine haematologic and biochemical investigations were performed before and 1 to 2 days after ALA ingestion (longer if any abnormalities were found).

10.3.2 Results

Complete epithelial necrosis was present in all cases. No patient had cutaneous photosensitivity for longer than 2 days. Patients reported experiences ranging from mild discomfort to severe pain during the time of irradiation, in some cases requiring more analgesia than had been anticipated. After PDT, the treated area became inflamed within a few hours. This was followed by sloughing of the superficial layers after 1 to 2 days, leaving a shallow ulcer. Oral analgesics were usually required from the first or second day after PDT for approximately 1 week. There was no evidence of scarring in those lesions that had not undergone previous surgery. Some benefit was observed in only one patient, who became tumour free (with persistent mild dysplasia). The other two patients were unable to be evaluated because they had to go on to further treatment for metastatic neck disease and progressive tumour.

Patient No	Age/Sex	Site	Previous treatment	Histology healed/wk	Size of lesion before/healed	Histology longest follow-up/wk
1	85/F	P	Field change*	Mild (4)	4.6cm ² /0.8cm ²	Mild (76)
2	87/F	T	Field change*	N	4.0cm ² /0cm ²	NA [†]
3	65/F	BM/A/P/RM	Field change* 0.7	SCC (12)	8.5cm ² /8.5cm ²	NA [‡]

BM: buccal mucosa; P: Palate; T: tongue; N: no clinical disease macroscopically, but biopsy refused; NA: not applicable; A: alveolus. SCC: squamous cell carcinoma; RM: Retromolar trigone.

*All three patients with field change disease had multiple excisions of dysplastic areas and carcinomas over 5-12 years before photodynamic therapy.

[†]Developed metastatic neck disease.

[‡]Subsequent photodynamic therapy with a different sensitiser.

Full-thickness epithelial necrosis and sloughing present in all cases.

Patients 2 and 3 received 100J/cm²; patient 1 received 200J/cm².

Table 10.2: ALA PDT in field change disease

At inclusion, all patients were NOM0

10.3.3 Conclusion

There seems little point wasting much time discussing ALA in field change disease. Clearly given our current state of knowledge, the depth of effect is too shallow making it an inappropriate treatment for this stage of disease except in dysplasia.

10.4 Foscan® PDT

Based on the histological effects of Foscan®, this would seem to be the best drug to treat field change disease. The only potential problem is scarring when extensive lesions are treated. The depth of PDT effect is up to 10mms, which should be sufficient to destroy dysplastic tissue. Of great concern, however, is the repopulation of treated areas. If this is from genetically damaged tissue, it is likely that recurrence will be inevitable. Mapping of such abnormalities is essential if treatment is to be successful. At present, while fluorescence and elastic scattering techniques are being developed, the best we are able to do is

treat obvious disease with a margin of clinically normal mucosa. The extent of this is purely arbitrary but 5 – 10mm is usually selected.

10.4.1 Materials and Methods

Eight patients were included in this study with a mean age of 57 years (30 – 65 years), all of whom had at least one histologically confirmed squamous cell carcinoma, all of whom conformed to the criteria of Warren and Gates (1932): histologically confirmed malignant primary neoplasms with each tumour being distinct. The total number of lesions was 16 (1-5 lesions per patient) and 6 patients had received extensive prior therapy. The study was approved by the Ethics Committee of the University College London Hospitals and treatment carried out with informed consent.

Photodynamic therapy: The mTHPC was supplied as crystals, which were dissolved in a solvent of polythene glycol, water and ethanol to yield a concentration of 4mg mTHPC per ml. The drug dose was 0.15mgs/kg body weight and this was administered intravenously through a filter with care being taken to avoid extravasation. Illumination was carried out 72 –96 hours post injection. The light source used was a copper vapour pumped dye laser emitting red light at 652nm (Dynamic Light Hornsby, Australia) and delivery was via a 0.4mm microlens system (PDT Systems, Santa Barbara, CA).

Treatment was carried out under local anaesthesia in all but one patient (patient 2) and treatment times were short ranging from 113 – 480s. The treatment area was marked with dots of crystal violet and included a margin of normal tissue of 1 cm. This allowed checking of the position to allow for patient movement. Power density was kept below 250mW/cm² to reduce thermal effects and where possible single treatment spots were used. This was not always possible due to the undulating contours of the mouth so it is possible there was some overlap of treatment sites. This was considered acceptable as it was felt important that at least the prescribed light dose be delivered. Escalating light doses were given from 5 – 20 J/cm² as detailed in 10.4.2.

Patients were kept in reduced light following drug administration with controlled re-exposure over 2-3 weeks.

10.4.2 Results

Pts	Age/ Sex	Site	Stage TNM	Previous treatmen t	Light dose	Histology (wks) healed	Size cm ² pre/p ost	Hist/FU (wks) longest F/U
1	30/M	T	T1	S	5J/cm ²	PD	1.5/0.1	S &RT (12)
2	54/M	LT LBM SP	T2 T2 T2	Nil Nil Nil	5J/cm ² 5J/cm ² 5J/cm ²	HK (26) Normal(26) PD	6.1/0 12.6/0 1.2/0.6	NCD (88) NCD (88)
		RBM	T2	Nil	10J/cm ² 20J/cm ²	PD NCD	0.6/0.3 2.4/0	NCD (21)
		RMA	T1	Nil	10J/cm ² 10J/cm ² 5J/cm ² 10J/cm ²	PD PD PD PD	12.6/7.1 7.1/7.1 3.1/1.2 1.2/1.2	PR NR NR NR
3	61/F	BM	T2	S	10J/cm ² 20J/cm ²	PD NCD	4.9/0.8 0.8/0	NCD(35)
		LA	T2	S	10J/cm ²	NCD	2.0/0	NCD(44)
		P	T1	S	10J/cm ² 20J/cm ²	PD NCD	1.2/0.03 0.03/0	NCD(35)
4	59/M	BM	D	S	5J/cm ²	NCD	24.3/0	NCD(89)
		A	T1	S	10J/cm ²	Hyperplasia	0.3/0	Mild ED(64)
5	65/F	P	T1	S,C,PDT	5J/cm ² 20J/cm ²	PD NCD	6.2/3.1 3.1/0	NCD(52)
		A	T1	S,C,PDT	5J/cm ² 10J/cm ² 15J/cm ²	PD PD PD	2/1.3 1.3/0.6 0.8/0.3	For further PDT
6	60/M	LFO M	Tis	S	10J/cm ²	Fibrosed mucosa(8)	9.6/0	NCD(24)
7	64/M	T	T1	nil	10J/cm ²	Mild ED	1.1/0	NC (84)
8	59/M	RM	T2	S,CY,RT	10J/cm ²	PD	1.2/0.8	Lost to F/U

Table 10.3: Foscan® PDT in field change disease

Key: T-tongue, **BM**-buccal mucosa, **A**-alveolus, **(S)P**-(soft) palate, **FOM**-floor of mouth, **RM**-retromolar, **S**-surgery, **R**-radiotherapy, **C**-chemotherapy, **CY**-cryotherapy, **D**-dysplasia; **HK**-hyperkeratosis; **PD**-persistant disease; **NCD**-no clinical disease, **NC**-no change; **PR**-partial response, **NR**-no response, **ED**-epithelial dysplasia, **L**-left, **R**-right.

Light Dose	Dysplasia	Tis	T1	T2
5J/cm ²	1 CR		4 PD	2CR 1PR
10J/cm ²		1 CR	2CR 2PR 1NR	1 CR 1PR 1NR
15J/cm ²			1PR	
20J/cm ²			2CR	2CR

Table 10.4: Response rates with escalating light dose.

Key: **CR**—complete response, **PR**—partial response, **NR**—no response

Eight patients with field change disease were treated on this protocol. Within a few hours of treatment, the treated area showed erythema, oedema and blistering. This was followed by the formation of a mucopurulent slough which healed gradually over 1-2 months. Some of the patients who achieved a partial response at low light doses were re-sensitised and treated at a higher light dose Table 10.3

As can be seen from the results in tables 10.3 and 10.4, the low light doses of 5 and 10J/cm² were insufficient to clear the disease and it was only at higher doses of 20J/cm² that one could be confident of disease elimination. Of course this has the potential to cause some scar formation although this was only seen in 1 case in this series.

10.4.3 Complications

All 8 patients experienced pain on administration of Foscan®. This was occasionally severe, but settled fairly quickly. It is our current practice to administer a small amount of 0.5% lignocaine before injecting mTHPC. Treatment was well tolerated although 2 patients required local anaesthesia. Post-operative pain was unpredictable, but could be severe. The onset coincided with the ulcerative phase and subsided with healing. Opiate analgesia was used by most patients for a period of about 2 weeks. Scarring

was seen in one patient (6) who had a degree of tongue tethering. This was in part due to previous surgery that included a skin graft in the floor of mouth.

There were no biochemical or haematological changes following treatment and two patients had mild photosensitivity reactions following sunlight exposure

10.4.4 Discussion

The treatment results are clearly better with higher light doses. $10\text{J}/\text{cm}^2$ was sufficient to clear in situ disease, but only 2 out of the 5 T1 tumours was cleared. Both the T1 and T2 tumours treated with $20\text{J}/\text{cm}^2$ were cleared, suggesting the need for higher light doses. In this study, the number of patients is small, however, in this difficult group, who often have no therapeutic alternatives, Foscan® PDT does seem to offer an alternative to extensive surgical intervention.

10.4.5 Conclusions

Foscan® PDT can be of value in the management of field change disease. Treatment times are short, allowing large areas to be treated in a reasonable time, phototoxicity can be a problem, but can be overcome by careful controlled re-exposure to light. Not all patients had a good outcome, but given that the majority of patients were referred following conventional treatment failure, that is hardly surprising.

10.5 Summary

These studies demonstrate the efficacy of PDT in the ablation of early invasive and pre-malignant lesions of the oral cavity. It may therefore be a viable therapeutic alternative to extensive mutilating surgery. While long-term follow-up is necessary to determine recurrence of pre-malignant and overtly malignant foci, unlike conventional techniques such as surgery or radiotherapy, PDT may be repeated without loss of normal tissue. Irreversible side effects such as those seen in salivary, bony and vascular tissues with radiotherapy are also avoided.

Clearly there are problems with all approaches to the management of field change disease. The neck remains a difficult area and this is probably best managed with conventional surgery and radiotherapy.

PDT, with ALA would seem to have too superficial an effect to be of clinical value. Photodynamic therapy with Photofrin® or Foscan® offers a viable therapeutic alternative in the treatment of multi-focal field cancerisation of the upper aerodigestive tract. Its use alone, or where a small area of tumour persists, with surgical excision, offers functional and cosmetic advantages over conventional treatment modalities for this difficult condition.

Chapter 11: Interstitial PDT for the Treatment of Advanced Head and Neck Cancer

11.1 Introduction

The treatment of advanced head-and-neck cancer continues to pose a major challenge. Although some early cancers are cured effectively, the overall outcome remains unsatisfactory and the overall survival rates have remained unchanged for several decades (Black et al 1997, Parker et al 1997, Pisani et al 1999). About 30-40% of patients with head-and-neck cancer have persistent or recurrent locoregional disease after completion of definitive treatment.

Therapeutic options for these patients are often limited by previous radiotherapy or surgical procedures. In the majority, surgical salvage is either not feasible or carries a high risk of complications due to fibrosis generated from previous treatment or tumour invasion into vital structures (Ridge 1993, Watson et al 1998). Similarly, re-irradiation is often impossible and only a very small number of patients with recurrent disease treated by full-dose re-irradiation are long-term survivors. Also, the incidence and severity of late toxicity is markedly increased in comparison to that observed after the first irradiation (De Crevoisier et al 1998, Stewart 1999).

In the clinical setting, many studies have confirmed the treatment efficacy of PDT on several types of human cancers, emphasizing the potential for improved functional and cosmetic outcome in a wide group of patients, while achieving comparable tumour control (Dougherty et al 1998, Hopper 2000).

One of the main limitations of PDT is the penetration of light in tissues and although it is possible to obtain good palliation of advanced disease with surface illumination, theoretically at least, clinical benefit is only likely to be seen in tumours that can be completely illuminated. This imposes an approximate maximum depth of effect of 1cm with current drugs in clinical use. The use of photosensitisers with a greater red shift such as bacteriochlorins (activation wavelength 730nm) should result in a greater depth of effect but wavelengths above 750 – 800 nm produce insufficient photon energy to trigger a PDT effect. However, interstitial treatment could overcome most of the constraints of

superficial treatment. Multiple fibres can be inserted directly into tumours under image control and large volumes of tumour can be destroyed in sites that are inaccessible to surgery or where surgery would cause damage to vital adjacent structures (Bown et al 2002, Nathan et al 2002). In this study, the safety and efficacy of interstitial PDT (IPDT) as a salvage treatment for recurrent head-and-neck cancers is investigated.

11.2 Materials and Methods

11.2.1 Patients

Between July 1997 and December 2002, a total of 45 patients with recurrent head-and-neck cancers were treated with salvage IPDT. Six patients were excluded from analysis because of insufficient data. Of the remaining 39 patients (61 treatments), 25 were men and 14 were women and their median age was 58 years old (range 8-84) (Table 11.1). Thirty-six patients (92%) had received prior radiotherapy, either alone or adjuvant to surgery or combining with chemotherapy as the primary treatment (35/36) or as a salvage treatment (1/36). Among those who received radiotherapy as part of their primary treatment, 25/35 had external irradiation only, 3/35 had both external irradiation and brachytherapy, whilst the other 7/35 had concurrent chemotherapy. Fifteen patients (38%) received surgery, either alone or as part of the combined primary treatment modalities. Sixteen patients (41%) received prior salvage treatment, including surgery (12/16), brachytherapy (3/16) and palliative chemotherapy (3/16), after recurrence was encountered. Table 11.1 summarizes the details of patient characteristics.

Median age	58 years (range 8-84)
Sex	
Male	25(64%)
Female	14(36%)
Primary treatment	
Surgery	15(38%)
Radiation	35(90%)
External only	25
External & brachytherapy	3
Concurrent chemotherapy	7
Salvage treatment after recurrence noticed	16 (41 %)
Surgery	12
Brachytherapy	3
Palliative chemotherapy	3

Table 11.1: Patient characteristics

All patients were referred because the recurrent tumours were either considered unresectable or the patients were not suitable for further surgery due to their general condition and were not candidates for further irradiation. Thus all the patients selected in this study were regarded as having a poor prognosis.

The sites and pathology of the recurrent tumours were diverse. Almost every anatomic sub-site of the head and neck region was involved (Table 11.2). Squamous cell carcinoma was the most common pathology (27/39, 69%) whilst other tumours like adenoid cystic carcinoma, olfactory neuroblastoma, follicular cell carcinoma, hemangiopericytoma, branchiogenic carcinoma, primitive neuroectodermal tumour and sarcomas were also encountered. The stage of recurrent tumours was very advanced, rT4, rN2 or rN3, in 85% of the patients (33/39). Recurrent T₃ and T₂ diseases were also noted in 4 and 2 patients, respectively. Table 11.2 summarizes the details of tumour characteristics.

	No of patient sites
Anterior tongue	6
Buccal mucosa	2
Floor of mouth	4
Alveolar ridge	3
Hard palate	3
Tonsillar region	5
Tongue base	1
Oropharyngeal wall	2
Maxillary sinus	2
Nasal cavity	3
Nasopharynx	1
Parotid	2
Thyroid	1
Neck	4
Stage	
rT2	2
rT3	4
rT4	31
rN2	1
rN3	1
Pathology	
Squamous cell carcinoma	27
Adenoid cystic carcinoma	3
Basal cell carcinoma	1
Olfactory neuroblastoma	1
Follicular cell carcinoma	1
Hemangiopericytoma	1
Branchiogenic carcinoma	1
Primitive neuroectodermal tumour	1
Osteosarcoma	1
Malignant fibrous histiocytoma	1
Alveolar soft part sarcoma	1

Table 11.2: Tumour characteristics

Pre-treatment evaluation included a complete physical examination, chest X-ray, complete blood cell count, and biochemical profile. A tissue diagnosis was obtained from all patients to confirm the recurrence of disease. All patients received either computed tomography (CT) or magnetic resonance imaging (MRI) to assess the extent of tumour and to determine the stage of recurrent disease. The experimental nature of the treatment was thoroughly explained and all patients gave informed consent.

11.2.2 Interstitial Photodynamic Therapy

Patients were sensitised with 0.15 mg/kg intravenous meso-tetrahydroxyphenyl chlorin (Foscan®, Biolitec Pharmaceuticals Ltd), kept in reduced room lighting to prevent skin photosensitivity and treated 4 days later. The light source was a diode laser (Diomed or CeramOptec, wavelength 652nm) with its primary beam feeding into a beam splitter to produce 4 treatment fibres (core diameter 0.4mm, bare tip). The power of each treatment fibre was 100mW and a power-meter was used to ensure the power output at the tip of each fibre. 18 gauge needles were then positioned percutaneously into the tumours. Initially this was carried out freehand, but subsequently ultrasound-, CT-, or MRI-guidance was used. The treatment fibres were passed through the needles using a flagging system to ensure that the fibre tip was exposed to the tissues and not still inside the needle. Four fibres were positioned at a time and a pullback technique was used using 1cm steps to treat thicker tumours. The energy applied to each treatment “station” was 20J (energy at point sources is in joules, with no surface area, unlike surface illumination). Ten treatments performed in this study were under local anaesthesia whilst the other 54 treatments were under general anaesthesia. After the administration of the sensitiser, patients were protected from bright sunlight for a total of 3 weeks. They were exposed to light of 100 lux on the first day, increasing by 100 lux per day. At 14 days, they were allowed outside using sensible precautions against sunlight.

11.2.3 Assessment and Follow-up

Criteria for response were as follows: a complete response (CR) was defined as complete regression of all evidence of disease; a partial response (PR) required

a 50% decrease of the summed products of the two largest perpendicular diameters of all measurable lesions, without an increase in size of more than 25% in any lesion or the appearance of new lesions; stable disease (SD) was defined as no significant change or any change in tumour size that was less than a partial response but not large enough to be considered progressive disease; progressive disease (PD) was defined as an increase of at least 25% in the size of measurable lesions or the appearance of any new lesion.

Treatment effects were assessed 4 weeks after IPDT and the responses were monitored using post-treatment scans. Where possible, these were of the same type as the pre-treatment scans to facilitate direct comparison. Patients with partial or complete responses were followed-up every month for 6 months then every 3 months thereafter. In addition to the quantitative measurement of tumour response, a qualitative measure was also used. Because the tumour extent in most patients of this study was very extensive, most treatments were carried out for palliative intent, thus, an index symptom was identified and benefit was assessed in relation to this response. For most of the patients, treatment objectives were often very limited although 7 patients were treated with curative intent (Table 11.3).

	Number of patients	Achieved
Cure	7	3(43%)
Palliation		
Swelling	26	18(69%)
Pain	6	3(50%)
Bleeding	3	3(100%)
Dyspnoea	2	1(50%)
Aphonia	1	1(100%)
Brachial Plexus Compression	1	1 (100%)

Table 11.3: Treatment Objectives

11.3 Results

The median overall (intent-to-treat) survival time of the patients was 14 months (Figure 11.1). A total of 61 treatments were performed in this study. Twenty-

four patients (62%) received only a single treatment; ten patients (26%) received two treatments, four patients (10%) received three and one patient received five treatments. For those who received treatment more than once, the median interval between each treatment was 6 months (range 1-16 months). The response of IPDT could be assessed in 56 treatments. Among them, CR was achieved in 8 treatments (14%); PR was achieved in 42 treatments (75%), SD was noted in 4 (7%) whilst PD was encountered in 2 treatments (4%).

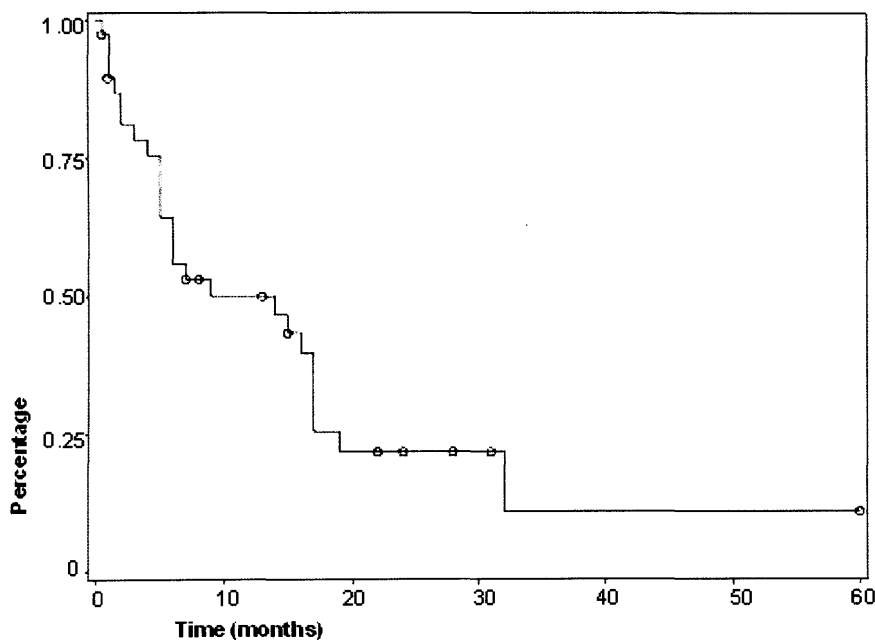


Fig 11.1: Overall survival (Kaplan-Meier curves)

The median disease-free survival time was 22 months (Figure 11.2) whilst the overall response rate (CR + PR) was 89%. The local control rate at 6 and 12 months were 54% and 41%, respectively. The age and sex of patients; modality of prior treatment; tumour site, pathology and stage did not correlate with the survival of the patients.

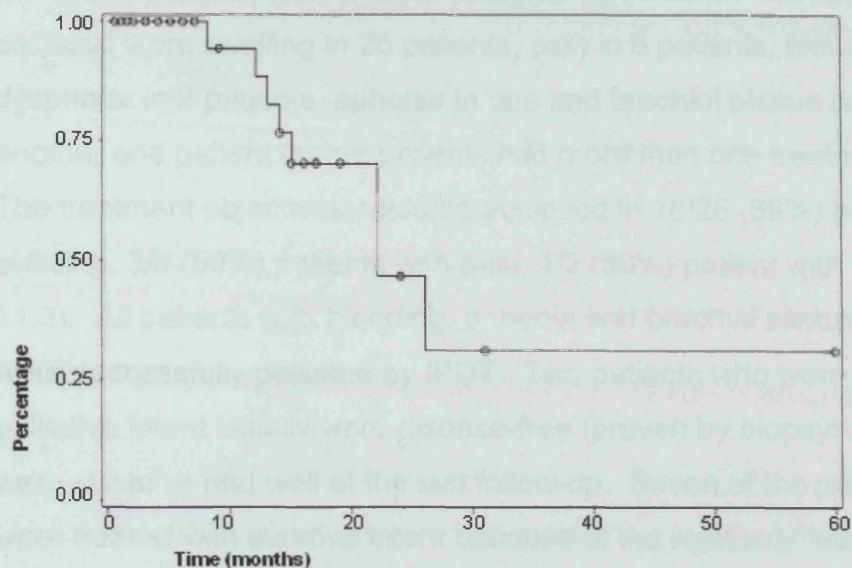


Fig 11.2: Disease-free survival (Kaplan-Meier curves).

However, survival benefit was observed in patients whose response of initial treatment was CR or PR (responders in the initial treatment). They had significant better survival time than the non-responders (Mantel-Cox log-rank test, $p < 0.01$; Figure 11. 3).

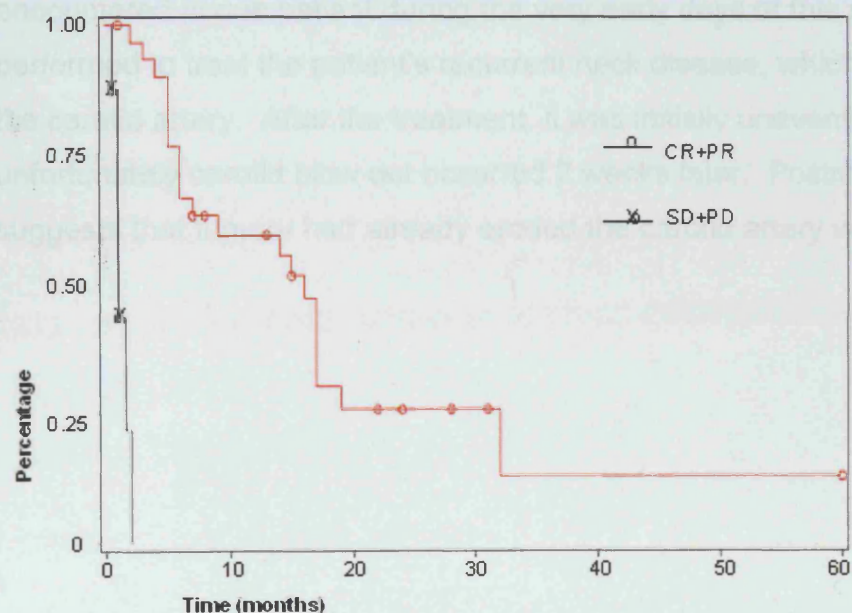


Fig 11.3: Cumulative survival (Kaplan-Meier curves) of responders and non-responders to their initial treatment.

Thirty-two patients were treated with palliative intent. The symptoms to be palliated were swelling in 26 patients, pain in 6 patients, bleeding in 3 patients, dyspnoea in 2 patients, aphonia in one and brachial plexus compression in another one patient (some patients had more than one treatment objective). The treatment objectives could be achieved in 18/26 (69%) patients with swelling, 3/6 (50%) patients with pain, 1/2 (50%) patient with dyspnoea (Table 11.3). All patients with bleeding, aphonia and brachial plexus compression were successfully palliated by IPDT. Two patients who were treated with palliative intent initially were disease-free (proven by biopsy) after IPDT and were still alive and well at the last follow-up. Seven of the patients in this study were treated with curative intent because of the relatively “early” stage of recurrent disease (rT₂, rT₃ and one extensive but superficial rT₄). Among them, three patients are still disease-free to date (Table 11.3).

Post-treatment tissue swelling and pain were noted in most of the patients but would usually subside within 2-4 weeks. Skin photosensitivity was noted in only one patient who failed to comply with the light regime, exposing himself to outdoor natural light early in the photosensitive period. The only major treatment-related side effect in this series was carotid blow out. It was encountered in one patient during the very early days of this study. IPDT was performed to treat the patient’s recurrent neck disease, which was very close to the carotid artery. After the treatment, it was initially uneventful, but unfortunately carotid blow out occurred 2 weeks later. Postmortem materials suggests that tumour had already eroded the carotid artery wall (Fig 11.4).



Fig 11.4: Cytokeratin antibody staining of tumour on the internal wall of the carotid artery. Postmortem finding 14 days after PDT

Since then, ultrasound-, CT-, or MRI-guided insertion of treatment fibres (Figure 11. 5) has been routinely performed to avoid injury to vital organs.

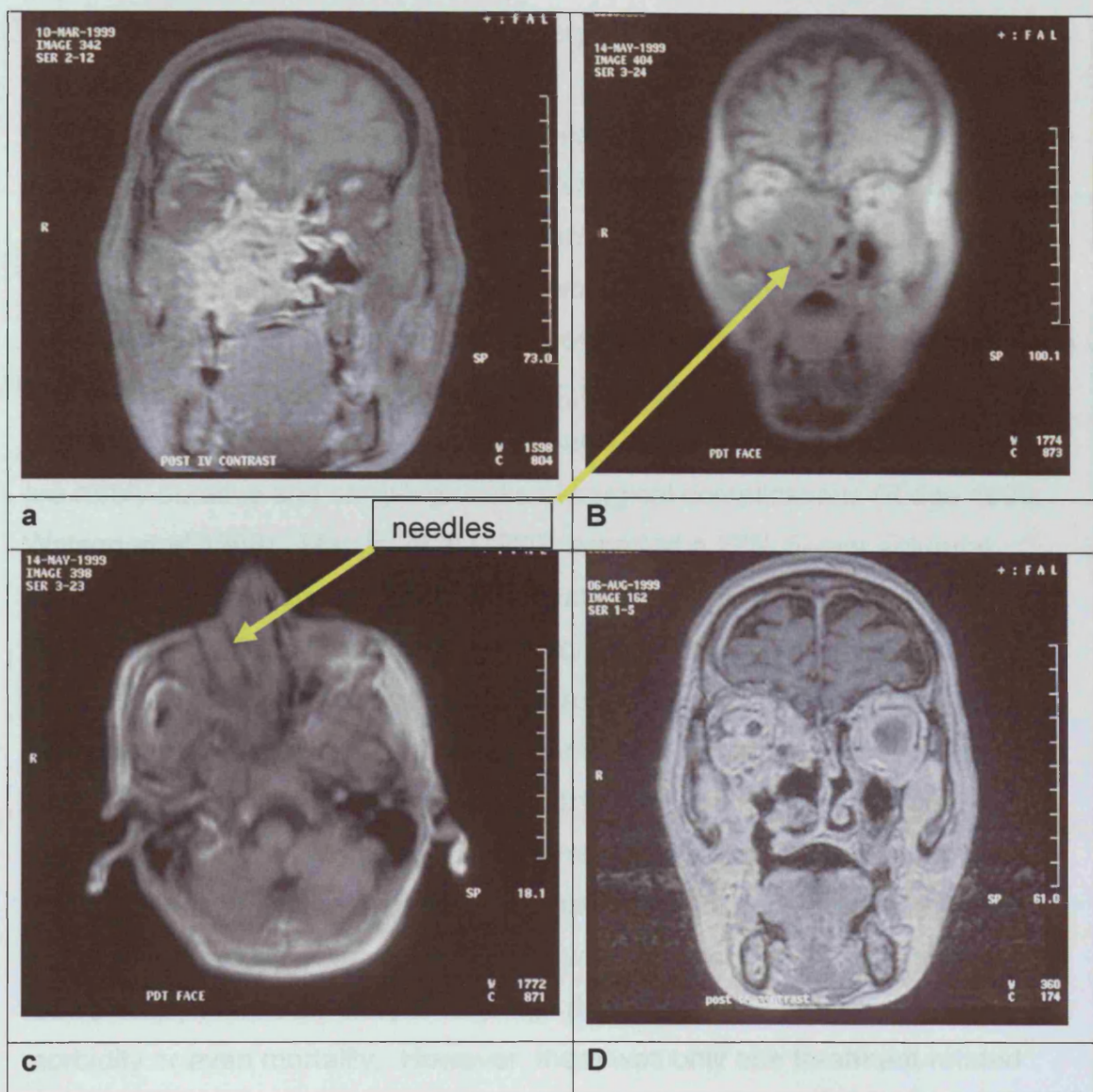


Fig 11.5: a - Recurrent adenoid cystic carcinoma of the right maxilla. Symptoms of epistaxis and nasal obstruction. b and c showing titanium needle placement under MR control. d - treatment response resulting in relief of epistaxis and obstruction. Note visualisation of needles by artefact only

11.4 Discussion

Despite the advances in oncological disciplines during the last decades, most of the recurrent head-and-neck cancer patients still succumb to their disease. There are many different treatments available including surgery, re-irradiation and chemotherapy; each has certain benefit to limited groups of patients. But generally, to re-treat these patients with any existing modality carries a significant morbidity without much survival advantage (Ganly et al 2000).

Among these treatment modalities, surgery is frequently employed to salvage recurrent head-and-neck cancer patients. It was also observed in this study that 12 of the 16 patients who had received prior salvage treatment had salvage surgery (Table 11.1). However, surgical salvage of local recurrence is dependent on the site and previous treatment of the primary tumour as well as the patient's general condition. It is known that recurrent glottic cancers that failed primary radiotherapy and are still confined to the laryngeal framework can be treated by surgery with curative intent (Nibu et al 1997, Maipang et al 1989). But larger recurrent tumours over other sub-sites of the head-and-neck region are rarely curative and carry high risks of surgical complications (Ridge 1993, Watson et al 1998). Marcial et al (1980), reported a 27% 5-year actuarial survival from oropharyngeal cancer patients who undertook surgical salvage (Marcial et al 1980). However, serious complications occurred in 21% of their patients. Rate et al (1991), combined surgical resection with intraoperative radiotherapy to salvage recurrent head-and-neck cancers and they achieved a 54.9% 2-year survival rate (Rate et al 1991). But again, perioperative mortality was 8.5% and severe complication occurred in 32.5% of the remaining patients. In the present study, the majority of our patients were considered non-surgical candidates because of tumour extent as well as history of previous surgery and radiotherapy that could carry a very high risk of peri- and post-operative morbidity or even mortality. However, there was only one treatment-related mortality in the very early days of this study and it was successfully prevented in future patients by the use of image-guide treatment. There was no peri- or post-treatment morbidity or complication and photosensitivity was also not a problem as long as the patient could comply with the light regime. Thus, IPDT seems to be a relatively safe measure for surgically high-risk recurrent head-and-neck cancer patients.

Apart from its role as a main treatment modality for primary head-and-neck cancers, radiotherapy has been widely used in the treatment of recurrent diseases. Since most of the head-and-neck cancer patients have received radiotherapy in their primary treatment, the main concern for re-irradiation is the damage to the previously irradiated tissues that may result in more severe late complications. Many different radiotherapy regimens have been used; among them, external beam re-irradiation is the commonest form but better local

control could only be achieved when the radiation dosage is greater than 60Gy (Ganly et al 2000). A large-scale study using full-dose re-irradiation to treat unresectable head-and-neck carcinoma showed the overall survival at 2 years was 21% (median survival 10 months) (De Crevoisier et al 1998). However, the incidence and severity of immediate and late toxicity (including 5 carotid blow-outs) was markedly increased compared to that observed after primary irradiation (De Crevoisier et al 1998). Alternatively, lower doses of re-irradiation can be given with brachytherapy (Harrison 1997). With either “high-dose-rate” or “low-dose-rate”, brachytherapy has been shown to be effective in the treatment of recurrent head-and-neck cancers (Puthawala et al 2001, Krull et al 1999, Glatzel et al 2002). Using interstitial high-dose-rate brachytherapy, Krull et al (1999) reported a local control rate of 49% at 12 months (overall median survival time less than 12 months (Krull et al 1999) whilst Glatzel et al (2002), reported a 74% response rate and overall median survival was 6 months in patients with recurrent tumours (Glatzel et al 2002). Puthawala et al (2001), used interstitial low-dose-rate brachytherapy and achieved a 21.7% 5-years overall survival rate (Puthawala et al 2001). However, moderate to severe complications occurred in 27% of their patients. Radiotherapy is also notorious for causing tissue fibrosis and constrained blood supply that may interfere with the efficacy of further treatments. Despite the fact that the majority (92%) of our patients had prior irradiation (including 6 with brachytherapy and 7 with chemoradiation), the response rate to IPDT in the present study was 89%, the overall median survival time was 14 months and local control rate at 12 months was 41%. Five of our patients are alive without evidence of recurrence to date and no late complications have been noticed. Because of extensive variation in patient selection criteria and treatment methods employed, a judicious comparison of these studies is not possible. However, our results clearly demonstrated that IPDT could be effective in treating recurrent tumours that have failed radiotherapy (including external beam, brachytherapy and chemoradiation). The survival data presented herein also forecast an encouraging potential to use IPDT as part of combined treatment modalities for recurrent head-and-neck cancers.

Perhaps the most commonly used and investigated chemotherapeutic agents in recurrent head-and-neck cancers are cisplatin and 5-FU. Used either as a

single agent or in combination, the median survival is 6 months in both cases, although combination chemotherapy increases overall response rates from 10%-20% to 30%-40% (Clavell et al 1994, Forastiere et al 1998, Vokes 1996, Jacobs et al 1992, Forastiere 1994). A randomised trial comparing the effectiveness between CABO (cisplatin, methotrexate, bleomycin and vincristin), PF (cisplatin and 5-FU), and cisplatin alone in recurrent head-and-neck cancers showed similar response rates for CABO (34%) and PF (31%) but better than cisplatin alone (15%) (Clavell et al 1994). However, there was no difference in overall survival between the three arms. New chemotherapeutic agents have also been tried in recurrent head-and-neck cancers. Unfortunately, the major response rates were not significantly better than traditional cisplatin-based chemotherapy and the overall survival has not been improved (Catimel et al 1994, Forastiere et al 1998). It is generally agreed that results of chemotherapy in the treatment of recurrent head-and-neck cancers show only moderate response rates and these are of short duration producing little or no increase in survival. Compared with chemotherapy, IPDT not only achieves a better response rate (89% response rate) but also significant survival benefit (overall median survival 14 months), especially in those who were responders to their initial treatment. Thus, IPDT could be a reasonable alternative in the palliation of recurrent head-and-neck cancers.

It is agreed that improving the patient's quality of life (QOL) is a very important goal in palliative cancer treatments. Consensus concerning the definition and measurement of QOL is difficult because of the complexity of the concept. There are few judicial and objective measurements (Aaronson 1990), but there are a number of different questionnaires used to investigate the QOL in head-and-neck cancer patients (Sherman et al 2000, Bjordal et al 1994, Nguyen et al 2002, Hammerlid et al 2001). Currently there are versions of the University of Washington and EORTC questionnaires specifically designed for head and neck cancer patients – for a comparison of these two see Rogers et al 1998. It is also difficult to evaluate patients' QOL in very advanced head-and-neck cancer patients if they are gastrostomy fed, as seen in most of our patients. As an alternative, treatment objectives could be specified and monitored as an indirect indicator of the patients' QOL (Schleicher et al 2001). In this study, we identified treatment objectives for each patient and evaluated if the symptoms

could be palliated with IPDT (Table 11.3). Generally, about 70% of the symptoms could be successfully palliated with IPDT. Compliance to the light regime was not a big problem to the patients because their daily indoor activities were not restricted and outdoor activities could be recommenced 2-3 weeks after treatment. Patients' willingness to be re-treated could also indirectly reflect their satisfaction in treatment and improvement in QOL. Thirty-eight percent of the patients in this study received more than one IPDT treatment. The real percentage of patients who were willing to repeat their treatment might be higher than that value because some of the remaining 62% patients didn't survive long enough to receive the second treatment (median interval between treatments was 6 months).

To improve the current limitation in the depth of tissue penetration with surface illumination PDT, newer photosensitisers were developed with longer activation wavelengths (Hsi et al 1999). It is known that the depth of light penetration increases with longer wavelengths of light. However, there is an inverse correlation between the wavelength and energy of light. Thus, it would appear there is an absolute maximum depth to which light of clinically useful wavelengths can penetrate. Alternatively, the limitation in tissue penetration could be overcome by interstitial treatment. Using a powerful photosensitiser Foscan®, which can produce effects to about 1cm, Nathan et al (2002), have shown that IPDT is a safe and effective measure for recurrent prostate cancer after radiotherapy (Nathan et al 2002). Animal studies also demonstrated that IPDT with Foscan® was sufficient to induce massive tumour destruction with minimal muscle damage (Andrejevic-Blant et al 1998). These results imply that IPDT with Foscan® could be a reasonable treatment option for tumours in unfavourable localisations in the oral cavity or pharynx that are surrounded by abundant muscles. Another advantage of interstitial treatment over surface illumination is that better light dosimetry can be achieved. With the improvement in imaging technology and facilities, we will be able to refine the light dosimetry to cover the whole extent of tumour with no or only minimal injury to the surrounding normal tissues. As a result, the treatment efficacy could be improved whilst complications could be minimised. A large-scale prospective study is warranted to verify this point.

11.5 Conclusions

Because of the complex regional anatomy and close proximity to vital structures, recurrent head-and-neck cancers are extremely difficult to treat. In this study, we have shown that IPDT could be an effective salvage measure for recurrent head-and-neck cancers. It is a minimally invasive procedure and can be used in carcinomas and sarcomas, in patients of all age groups, as well as in medically compromised patients that could not withstand complications of radiotherapy or may be too debilitated to undergo surgery. Thus, IPDT may serve as an important treatment option for the integrated head-and-neck oncology team.

Chapter 12: Future Developments of PDT

12.1 Drug Development

There are a number of limitations with current photosensitisers in clinical use and there are various ways of overcoming these.

12.1.1 Selectivity

So far, there has been little to suggest that a selective PDT effect – that is to say necrosis of tumour while preserving normal tissue from which a tumour has arisen – can be achieved with the treatment parameters and drugs in current use. All the current selectivity is purely as a result of the positioning of the light although ratios of photosensitiser in brain tumours compared with normal brain might prove an exception, but even in this site there are potential problems as normal brain is extremely sensitive to PDT even when concentrations of photosensitiser are low.

Work is currently being undertaken to see if it is possible to link an antibody fragment to a sensitiser (figure 12.1) and generate ratios of 100(tumour):1(normal). If successful this would dramatically increase PDT applications, especially in bladder tumours where good antibody fragments are available. This would overcome the problems of muscle damage and small volume bladder that restrict PDT application in this condition.

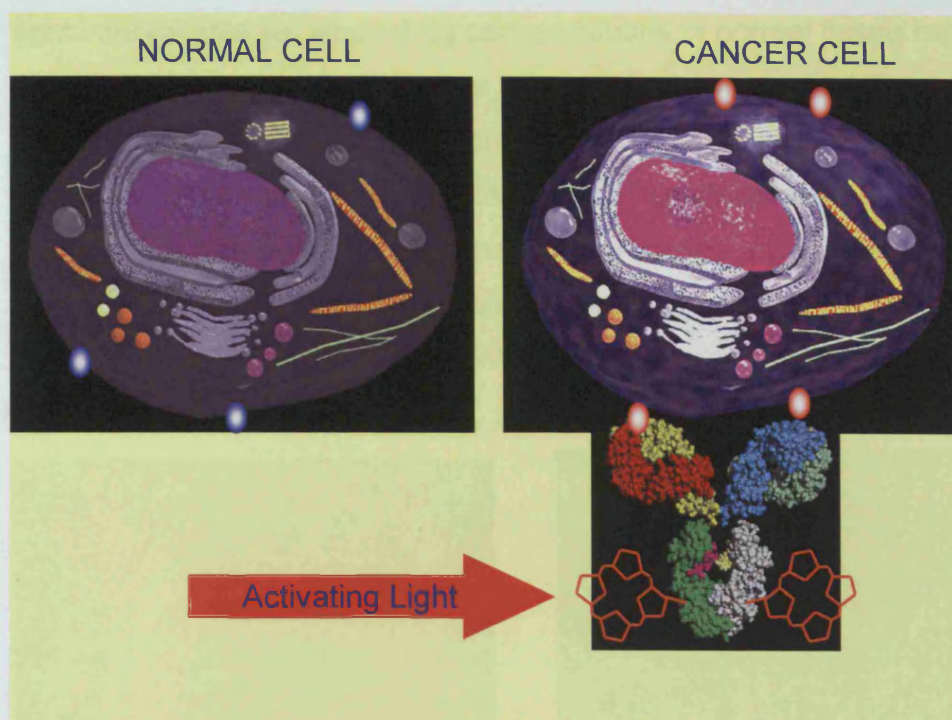


Fig 12.1: Principle of selectivity when specific antibody fragments are linked to photosensitiser resulting in increased selectivity of photosensitiser binding to tumour, (Photobiotics London UK)

12.1.2 Drug Light Interval

Most photosensitisers have an optimum drug light interval of hours to days before the recommended time of photoactivation. Clearly this is inconvenient for the patient and also makes the use of adjunctive intraoperative treatment difficult. If a surgeon is performing a neck dissection, then he or she would need to identify patients preoperatively when it is thought that it would be impossible to safely resect all the tumour. This is a major problem with drugs in current use as they all cause photosensitivity problems under operating lights. It would be much more useful to have a drug that could be injected systemically and activated in a matter of minutes at the end of an operation just prior to skin closure. One such drug under investigation is Tookad® which is being studied in prostate cancer.

There is also a great deal more to learn about optimisation of the drug light interval. All of the current treatment parameters were derived from animal experiments on drug kinetics and biodistribution. The idea was that the drug

light interval was set when drug concentrations in normal tissue had fallen, but were still high in the target tumour. Recent Russian studies (Stranadko et al 2003) on skin cancer suggest that this might be of academic interest, as it has been possible to successfully treat basal cell carcinoma with Foscan® at 24 hours (as opposed to 96hrs) with a greatly reduced drug dose and increased light dose (figure 12.2). The reduced drug dose also has an obvious beneficial impact on the duration of photosensitivity.

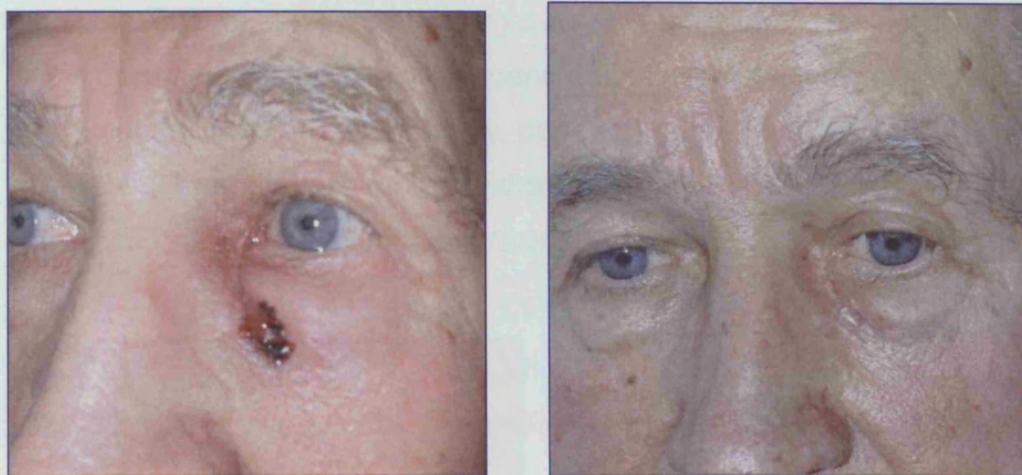


Fig 12.2: 78yr old male with 15mm diameter BCC treated with Foscan® PDT. Treatment parameters: Drug dose 0.04mg/kg DLI 24hrs Light 60J/cm² CR at 7 months (Stranadko et al 2003).

12.1.3 Drug Level Monitoring

There is obviously great scope for non invasive techniques to measure tissue drug levels in real time. Optical pharmacokinetics is one such potential method of doing this. The technique has certain similarities to elastic scattering spectroscopy (vide infra) and is at very early stage of development, however, it has the potential to revolutionise pharmacokinetic studies not only of photosensitisers, but also a number of other drugs. It is an intriguing prospect that future drug level measurements might be possible non invasively in real time.

12.2 Laser Development

Currently, nearly all treatments are carried out using light from laser sources. While this has proved successful, there are laser safety issues to consider and non laser light sources might be more widely applied. This is already the case in dermatology, where light emitting diodes (LEDs) and filtered Xenon light sources are widely used. One curious observation has been made using LED sources and that is a very large volume of tumour necrosis can be achieved by using low fluence rates and treating over several hours. This becomes practical in the treatment of tumours of the pancreas or liver with indwelling devices which can be placed at laparotomy, ported through the skin, activated for several hours and then simply withdrawn. One such system under investigation is the LED system from Light Sciences (figure 12.3).

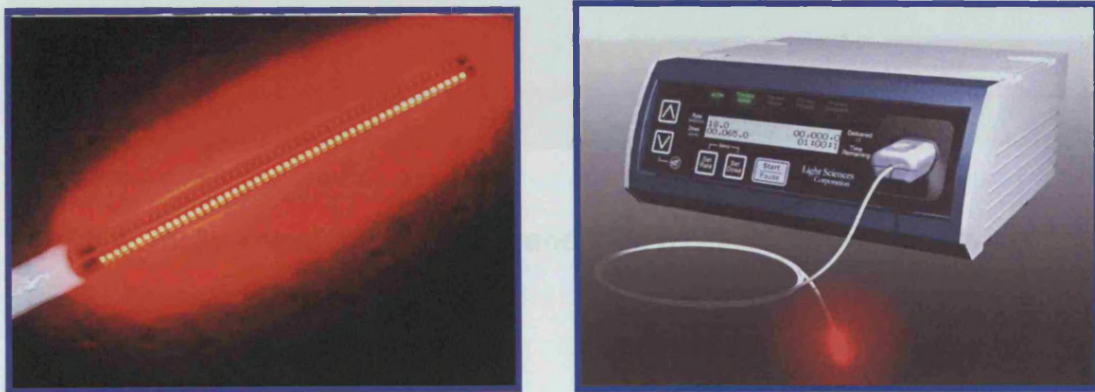


Fig 12.3: LED source for PDT treatment (Light Sciences Corporation Seattle US).

One other innovative approach to light delivery is BioLuminescence Activated Destruction of Cancer (BLADE or the “Firefly” concept). If light can be generated within malignant tissue, sensitisers can be activated and cause the cells to self destruct. This is achieved by dosing the administering D-luciferin which is cleaved by transfected luciferase previously introduced into the tumour attached to tumour specific antibodies. This is the mechanism by which fireflies produce visible light (520 – 540nm) but in this setting, rather than attracting a mate, results in lethal photosensitisation. (figure 12.4).

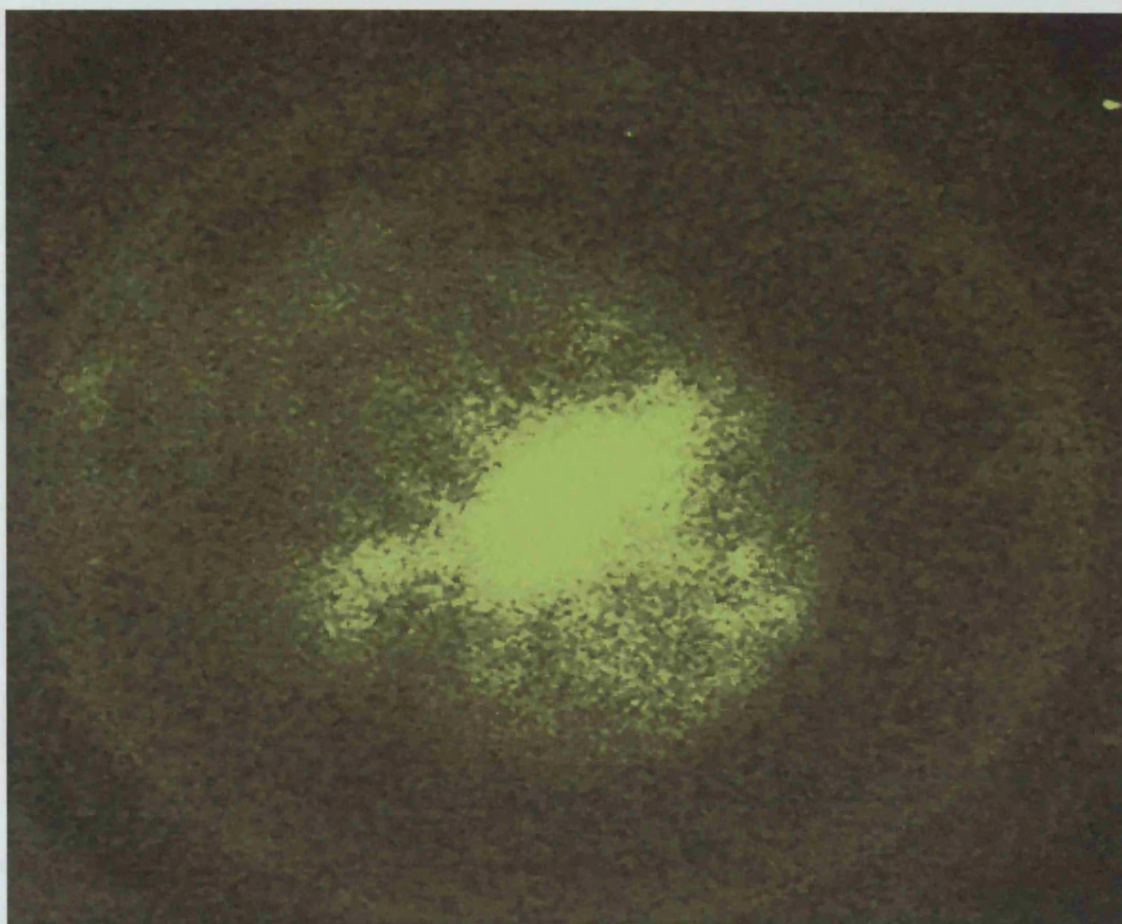


Fig 12.4: Bioluminescence of cells generated by luciferin/luciferase reaction (T.Theodossiou 2003)

12.3 Tumour Delineation

The crucial factor to give the best chance of curing any local disease such as oral cancer is comprehensive treatment of the whole tumour. This requires two things – accurate mapping of the extent of tumour and then ensuring the total disease volume is treated. This has a great deal in common with interstitial radiotherapy techniques, many of which can be applied here.

12.3.1 Surface Mapping

Histology remains the gold standard for tumour diagnosis, but there are a number of non invasive techniques that can be used to map tumours pre-treatment. This can be of great value when a tumour arises in a widespread

area of dysplasia. Detailed description of these techniques is outwith the scope of this thesis, however, the following seem to be applicable clinically.

12.3.1.1 Fluorescence Techniques

These rely on alterations in red/green fluorescence which correlate with increasing malignancy. These changes can be enhanced by the use of an aminolaevulinic acid mouthwash and improve the sensitivity and specificity of the technique.

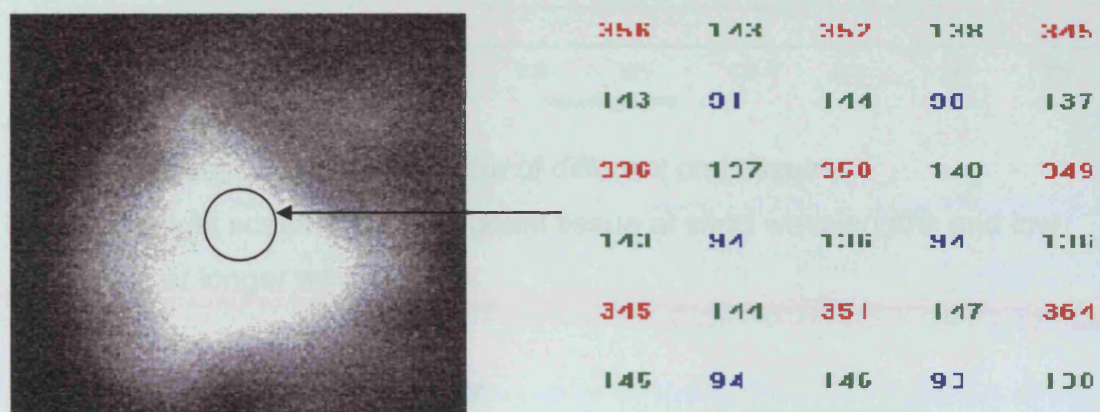


Fig 12.5: Enhanced fluorescence image and numerical readout

The numerical sample relates to clumps of approximately 5 cells within the white area of fluorescence – note red/green fluorescence ratio well above the normal 1:1 ratio – indicating malignancy. The blue numbers reflect background fluorescence.

12.3.1.2 Elastic Scattering (ESS)

ESS is a technique in which a short pulse of white light is fired into tissue and the returning signal, collected by a second immediately adjacent fibre, is displayed on a spectroscop. Intensity changes and alteration of gradients in the spectra have been shown to correlate well with surface tumours in vivo and in formalin fixed tissue.

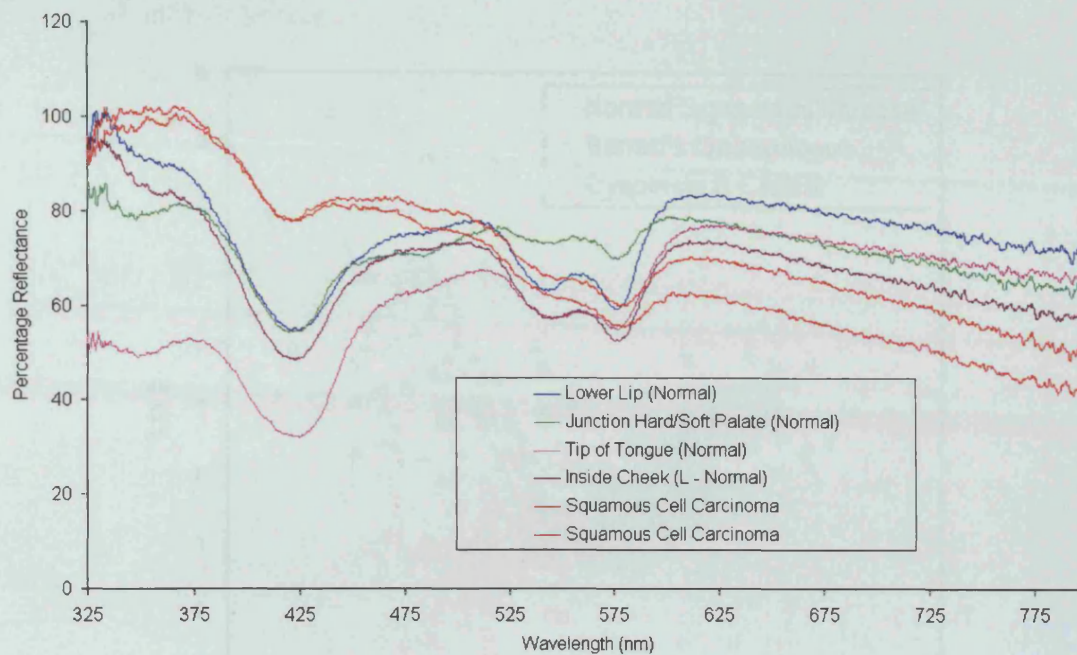


Fig 12.6: Elastic scattering spectra of different oral tissues

Note high light scattering of malignant tissue at short wavelengths and low scattering at longer wavelengths.

12.3.1.3 Raman Spectroscopy

This is an elegant technique that looks at changes in the wavelength of incident laser light as a result of tissue interaction. It has found application in Barretts Oesophagus and breast cancer (among others) but requires sensitive and expensive equipment as the signal intensity is weak.

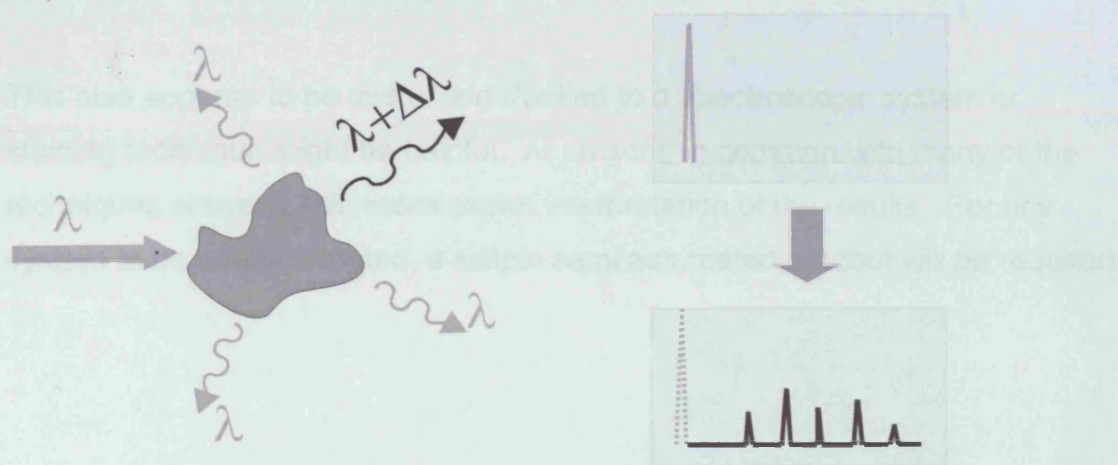


Fig 12.7: Example of diagnostic potential of Raman (vibrational) Spectroscopy (λ = incident photon $\Delta\lambda$ = wavelength shift after tissue interaction).

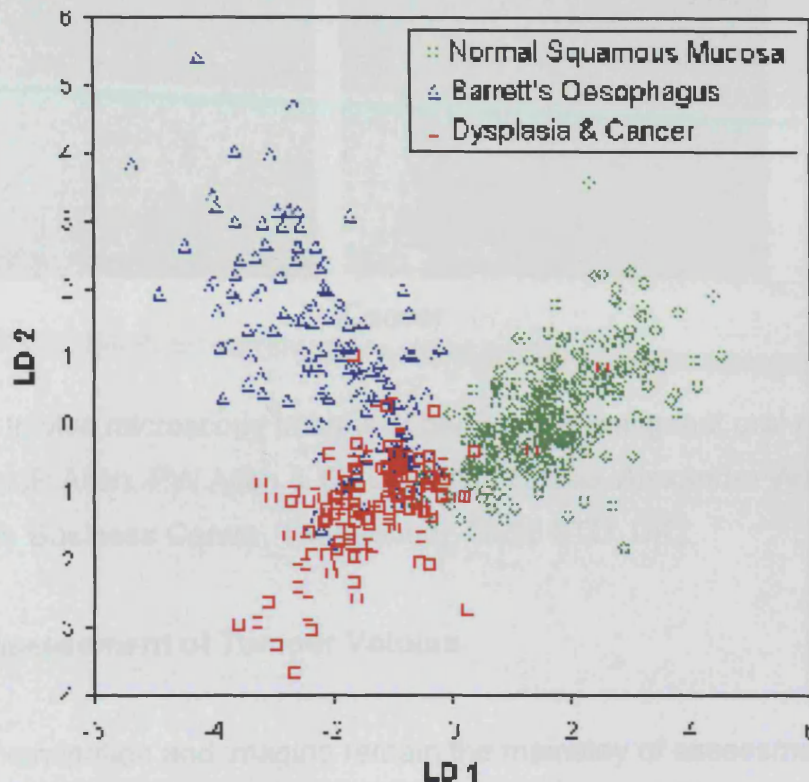
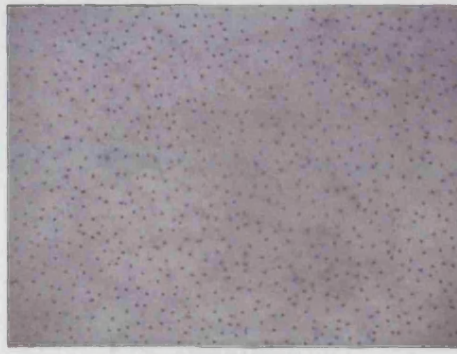


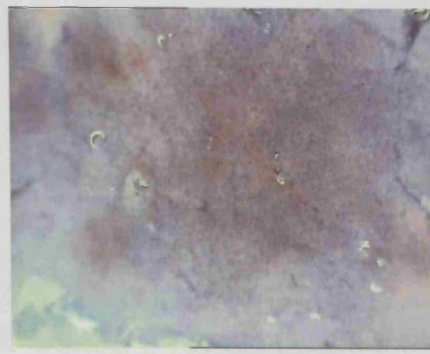
Fig 12.8: Scatter plot of Raman spectra in Barretts Oesophagus (Courtesy Dr Nick Stone, Head of Biophotonics Research Group, Principal Clinical Scientist/Senior Lecturer, Gloucester Hospitals NHS Trust, Gloucestershire Royal Hospital, Great Western Road, Gloucester, GL1 3NN).

12.3.1.4 In Vivo Microscopy

This also appears to be useful and if linked to a spectroscopic system or staining technique might be helpful. At present, in common with many of the techniques above, it still needs expert interpretation of the results. For any system to be widely adopted, a simple semi automated readout will be required.



Normal



Cancer

Fig 12.9: In vivo microscopy images of benign and malignant oral mucosa (Courtesy JP Allen, PW Allen & Co Ltd, Allen House, Alexandra Way, Ashchurch Business Centre, Tewkesbury, GL20 8TD, UK).

12.3.2 Assessment of Tumour Volume

Clinical examination and imaging remain the mainstay of assessment of tumour volume. Accuracy with these techniques has been discussed in earlier chapters, but they all have limitations.

12.3.2.1 High Frequency Ultrasound

This may be of value in determining tumour depth when treatment is planned with surface illumination. It appears that there is good correlation between ultrasound and histopathology. This can be easily applied to surface lesions such as skin tumours, but there is an obvious need in the head and neck for small probes suitable for evaluating oral tumours.

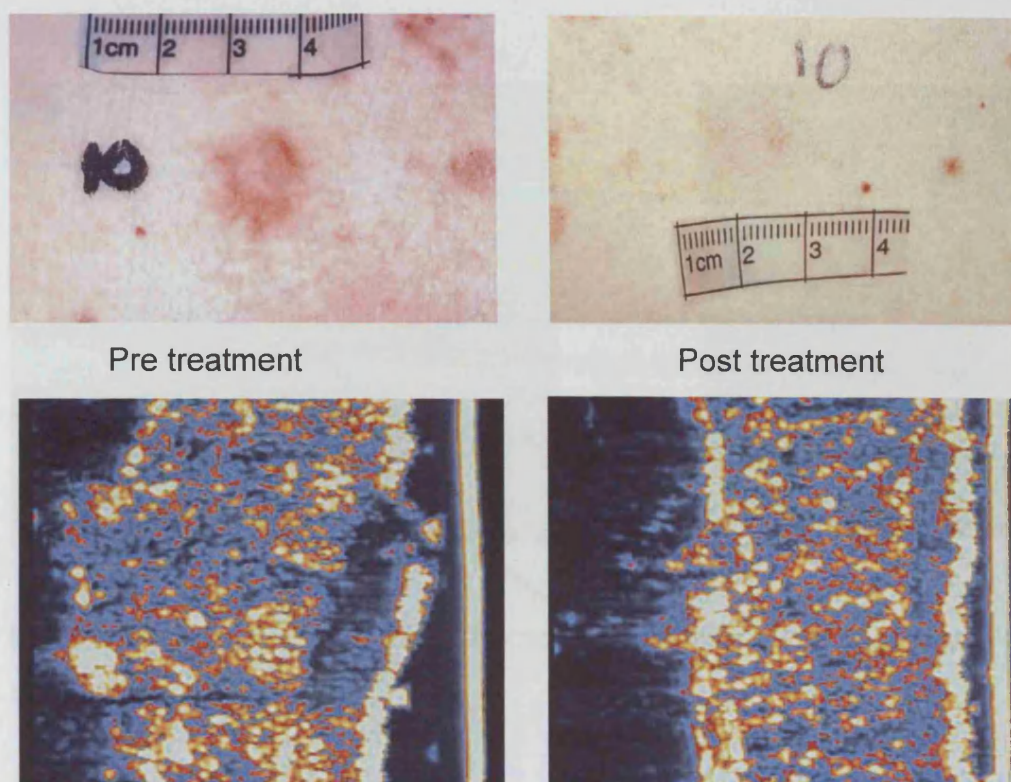


Fig 12.10: Clinical appearance of cutaneous BCC with time matched high frequency ultrasound before and after ALA PDT treatment (Photo courtesy of Dr A Sahota)

12.3.2.2 Functional Imaging Using Positron Emission Tomography

(PET scanning) might offer more information about tumour target volumes. It is still experimental, but it is relatively simple to compare PETCT data with CT scans to position treatment fibres at the site of glucose avid tumours (figure 12.11). There remains some doubt regarding the accuracy of these technique, so until it has been fully validated, it has to be cross checked with MR images. Co-localisation techniques – that is to say fusion of physiological and anatomical detail is currently in its infancy, but will almost certainly be more useful in the future.

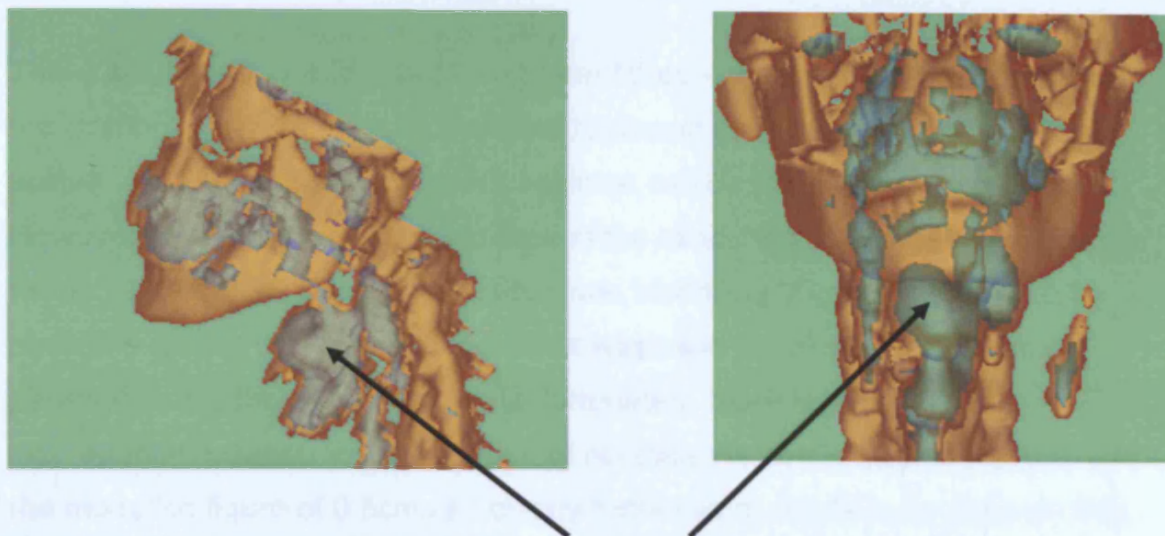


Fig 12.11: 3 Dimensional PETCT scan with glucose avid tissue identified at base of tongue

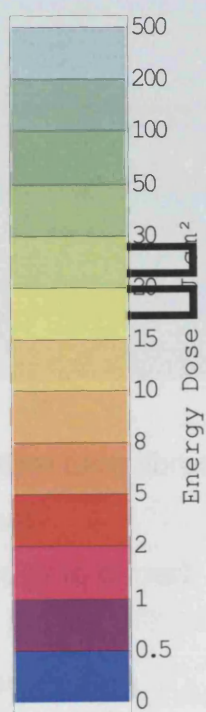
One of the main advantages of PDT over other treatments is the ability of normal tissue around a tumour to withstand the treatment and heal satisfactorily even when it has been included in a treatment margin. This often allows us to be slightly more radical with safety margins and depends on the type of drug used, for example, circumferential treatment of the oesophagus is well tolerated with ALA, but not with conventional Foscan® treatments (drug dose 0.15mg/kg, drug light interval 96hrs, 652nm light at 20J/cm²) which causes muscle damage and an unacceptable degree of stenosis.

12.4 Delivery Systems

Most published data on PDT concentrates on surface illumination techniques. While drugs like Foscan® produce clinically useful effects down to about 10 – 15 mm, the application of this therapy needs greater development in interstitial treatments. In Chapter 11, I have shown that this approach can be used effectively. However, there is still a great deal to be done to bring this therapy into regular practice.

12.4.1 Bare Fibres Versus Diffuser Fibres

There are clear advantages of using bare fibres – they are cheap, reusable and the distribution of light can be modified to almost any situation. Using a beam splitter and Foscan®, large tumour volumes can be treated in a short time. However, there is a great need to look at the exact distribution of light in human tissue. This can be done with Monte Carlo Modelling. Figures 12.12 – 12.15 show the results of one such model that was used to validate the treatment parameters we have been using. Unfortunately, there is a significant discrepancy between the 1cm radius of necrosis we see in clinical practice and the modelled figure of 0.5cms so clearly further work needs to be done on this.



The colour code for the diagrams of figures 12.12, 12.13, 12.14 and 12.15.

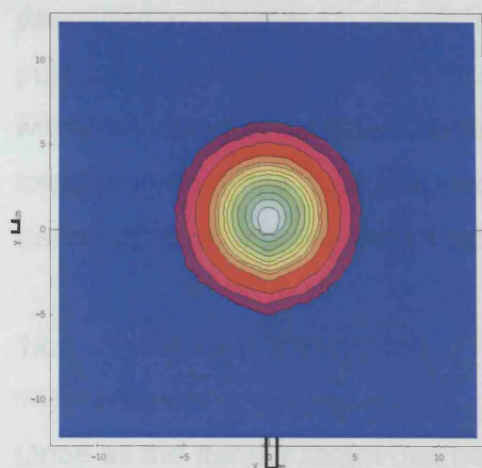


Fig 12.12: Bare Fibre:652 nm
(fibre perpendicular to paper)

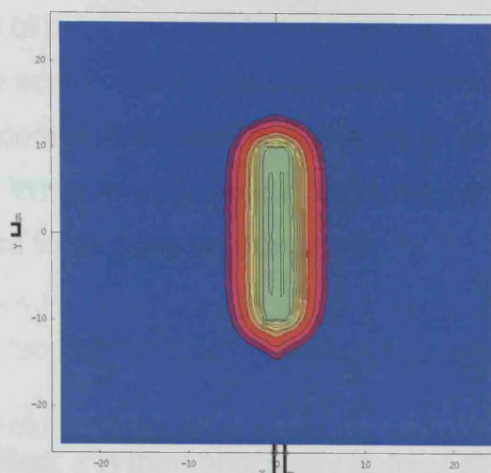


Fig 12.13: Diffuser fibre:652 nm
(fibre is in plane of paper)

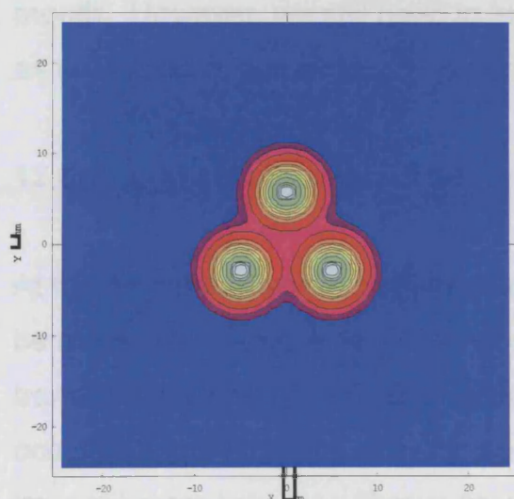


Fig 12.14: Interstitial bare fibres
in a triangle 652nm
(fibres perpendicular to paper)

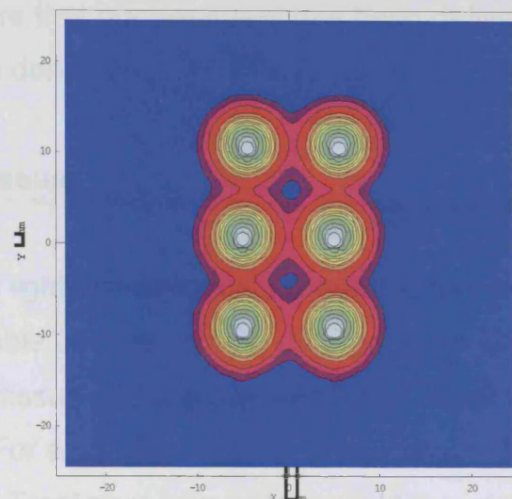


Fig 12.15: Interstitial bare fibre arranged
with pull back 652 nm
(fibres in plane of paper)

12.4.2 Fibre Positioning

The exact technique for fibre placement with interstitial treatment is open to debate. Radiotherapists with expertise in brachytherapy favour the use of plastic tube devices that can be loaded with diffuser fibres of variable length and computer generation of light dosimetry. This is clearly a very similar concept to afterloading techniques and has the potential to be quite accurate and effective. However, it is immensely time consuming and unlikely to be adopted by large numbers of oncology units. At the other end of the spectrum is the technique

described in Chapter 11, in which a series of point sources is used with a pullback technique. I suspect there will be some convergence of ideas, perhaps with positioning of catheters under image control, then removing the trocar and treating through the clear plastic cannula. While it is possible to undertake this treatment now, a great deal of work still has to be done on the detail.

12.5 Treatment Monitoring

Once all the tumour target has been identified, it is then necessary to treat all of it. This can be done using the surgical philosophy of treating all the disease with a surgical margin. PDT necrosis in normal tissue adjacent to tumour is acceptable as long as it heals satisfactorily and this seems to be the case in the mouth. However, we still need to ensure that our treatment has been delivered as we intend to our target. This can be done by:-

12.5.1 Light Measurement within Tissue

At the time the treatment is carried out, light transmission through the tissue can be measured in real time. This will enable us to overcome differences in light transmission through different types of tissue and compensate for changes that occur during the course of treatment. For example, vascular tissue seems to transmit more light than fibrous tissue. Treatment parameters could therefore be altered for different targets – for example a tongue tumour might require 20J/cm (for interstitial treatment with diffuser fibres) whereas prostate might require twice or three times this dose.

12.5.2 Measurement of Oxygen in the Tissues

Oxygen is consumed during PDT. This can be measured by continuous real time recording of in vivo HbSat levels during and after PDT by means of Visible Light Reflectance Spectroscopy. This spectrographic system calculates the changes in oxyhaemoglobin (HbO), and deoxyhaemoglobin (Hb) in $\mu\text{mol/l}$ from changes in the spectral attenuation derived from the light reflectance spectra acquired by the spectrometer over its 485-635nm detection range. From these values one can derive the total haemoglobin (HbT) in $\mu\text{mol/l}$ as the sum of the

HbO and Hb, and the haemoglobin oxygen saturation (HbSat, expressed as a percentage), which is HbO divided by HbT (Figure 12.16). There would seem little point continuing to treat when tissue oxygen levels are low and this kind of technique would help adjust treatment parameters such as treatment breaks to make them maximally efficient.

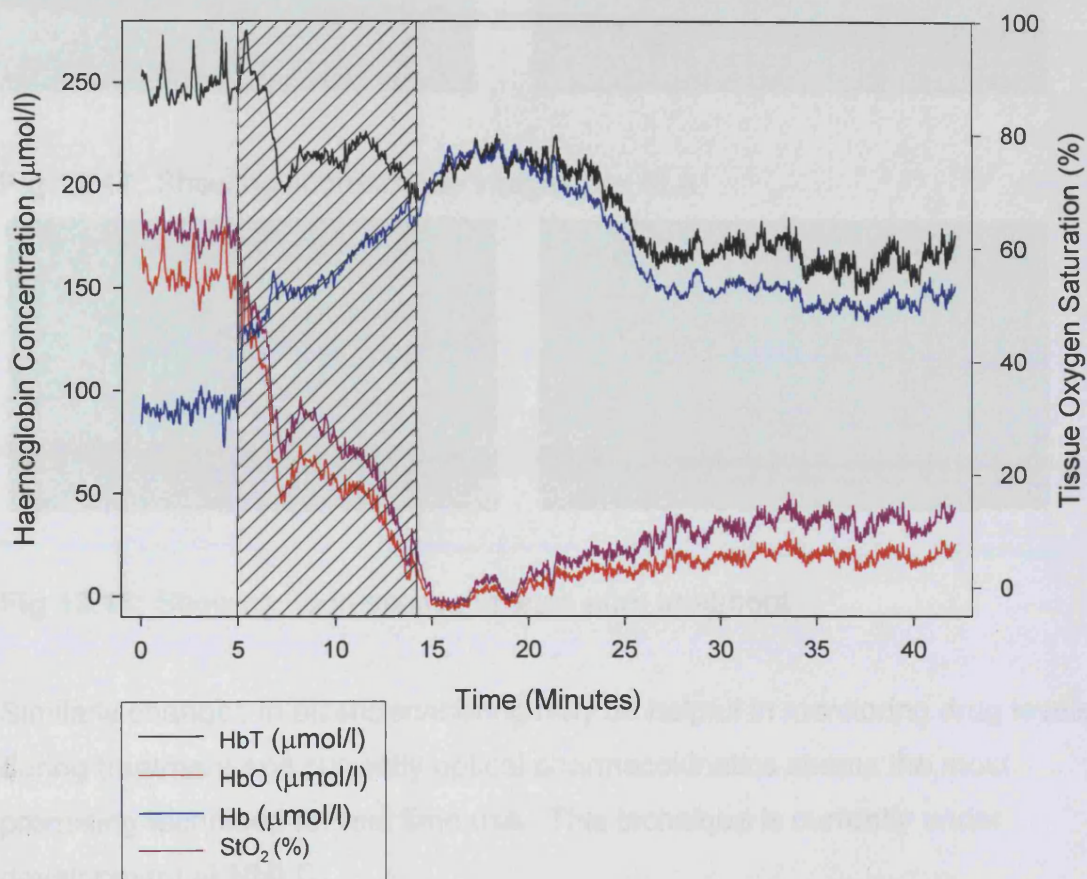


Fig 12.16: Experimental arrangement for the visible light spectrometer (VLS) with the PDT fibre and the VLS probe positioned on the surface of the liver at a fixed centre-to-centre separation (Woodhams J 2004)

12.5.3 Measurement of Active Drug in Tissues

It is possible to measure markers of drug in tissues – currently we can use a CCD camera to measure fluorescence during ALA PDT. (figures 12.17- 12.18). When the fluorescence pattern returns to presensitiser baseline, it may be that there is little point in giving more light. It is important to note that just because all the active photosensitiser (PPIX) has been photobleached, it does not

necessarily mean treatment is adequate, merely that there is no value in prolonging treatment.

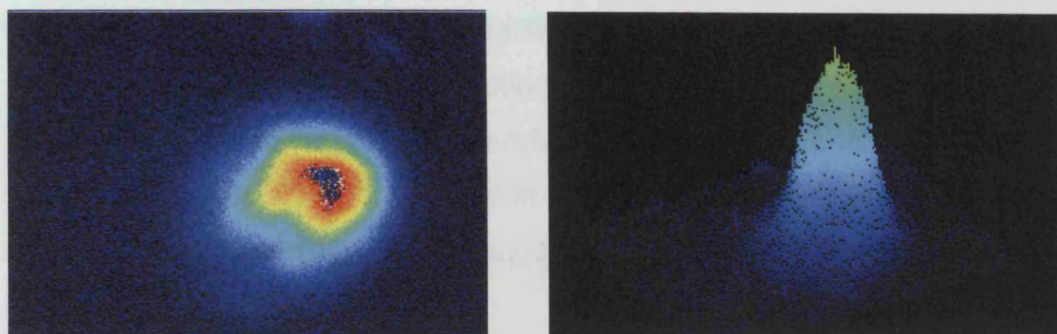


Fig 12.17: Showing fluorescence images pre ALA

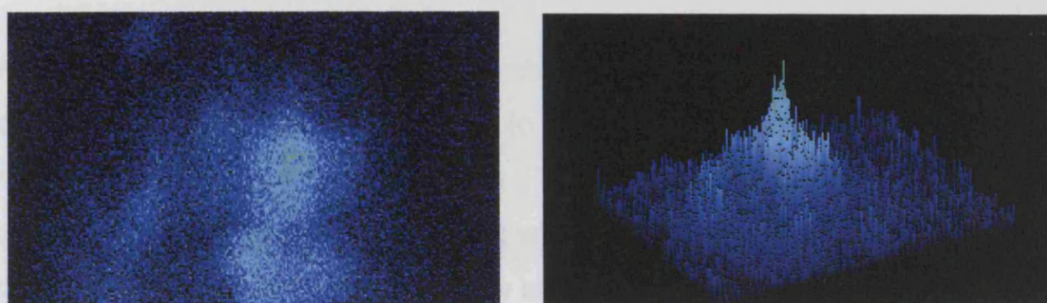


Fig 12.18: Showing fluorescence images after treatment

Similarly changes in elastic scattering may be helpful in monitoring drug levels during treatment and currently optical pharmacokinetics seems the most promising technique for real time use. This technique is currently under development at NMLC.

12.6 Combination Therapy

Most meaningful work on PDT so far is using it as a single therapy. Initially, this has been very important in order that we can work out the nature of the PDT effect. However, it might be that it will find a place as a therapy in combination with other techniques. Below is a series of possibilities – the list is far from complete and merely scratches the surface of possible future developments.

12.6.1 Hyperthermia

Apart from the obvious fact that heat kills tissue, all reactions tend to be accelerated by increasing temperature. The photochemical effect is no exception and combinations of heating with PDT result in greater treatment effects. There is already a considerable literature on hyperthermia and results look encouraging. The work has been going on since the early days of PDT and a variety of sensitisers have been studied (Levendag et al 1988, Yanase et al 2005).

12.6.2 Sensitiser Combinations

As shown in chapter 7, different sensitisers produce different effects and combinations of drugs may be used to optimise tumour ablation although not necessarily with improved selectivity. The same sensitiser can produce different effects over time in keeping with its pharmacokinetic characteristics, so that if treatment is carried out shortly after Foscan® sensitisation there might be a predominantly vascular effect, whereas if treatment were carried out at one day unwanted skin and muscle damage might be seen and these effects would be minimised if treatment were carried out at day five. Also, some sensitisers can be activated at different wavelengths, for example, ALA can be activated by red (635nm) or blue light (407-420nm) and Foscan® by red (652nm) or green light (511nm). These secondary absorption peaks might have different characteristics such that treatment effects are not too extensive around sensitive structures. For example, the treatment of Barrett's oesophagus with Foscan® activated by 652nm red light, would produce deep necrosis, whereas activation with green light of 511nm results in a more superficial effect that is still clinically useful in the ablation of dysplastic mucosa without the risk of stricture or perforation.

12.6.3 Photochemical Internalisation

There are a number of ways in which malignant cells develop resistance to cytotoxic drugs. One of these is to enclose the drug in organelles such as lysosomes and hence prevent them reaching the usual target (such as the

nucleus). PDT is capable of causing cell death, but in addition, caused sublethal cell damage around the necrotic centre. In this process, there is damage to intracellular organelles with release of the cytotoxic agent which is then free to act on the cell. This is demonstrated in figures 12.19 and 12.20. The first graph shows the effect of increasing adriamycin concentrations on adriamycin sensitive and adriamycin resistant cell lines. The resistant cell lines show a consistent cell survival fraction of 70%. If the experiment is repeated with PDT alone this figure falls to 50% but if the two treatments are combined it falls to 20%. Even this figure is higher than the true situation as the assay is a colourimetric (MTT) assay in which readings do never fall to zero.

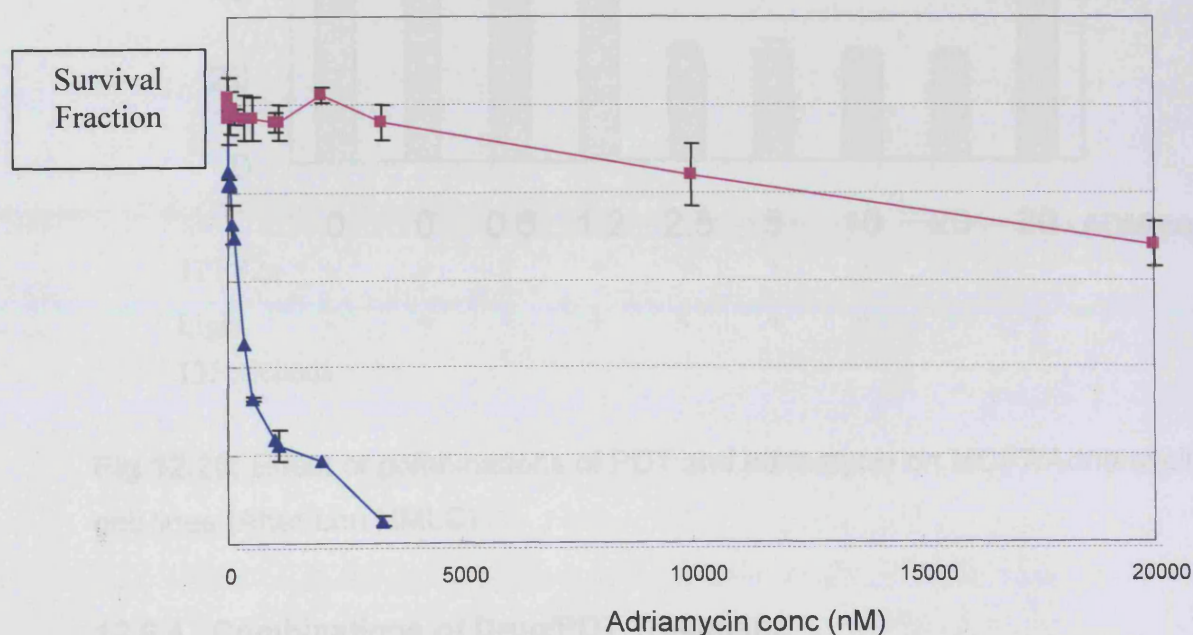


Fig 12.19: Effect of increasing adriamycin concentration on
MCF7 (sensitive) cell lines —▲—
MCF7/ADR (resistant) cell lines —■— (After Lou NMLC)

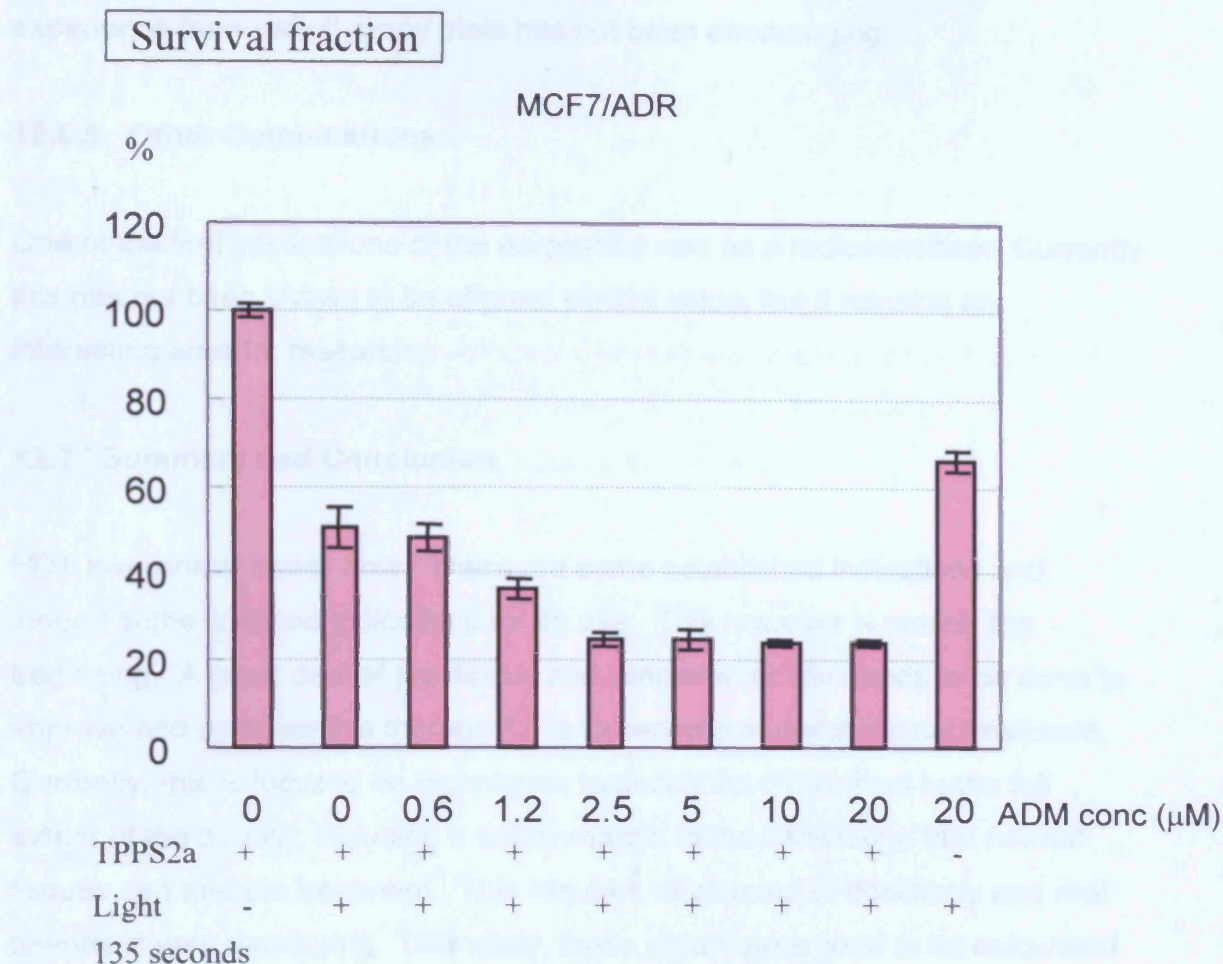


Fig 12.20: Effect of combinations of PDT and adriamycin on MCF7/Adriamycin cell lines (After Lou NMLC)

12.6.4 Combinations of Drug/PDT Treatment

Other therapies can be combined with PDT for example, antiangiogenic treatments such as Thalidomide and bioreductive agents such as RSU 1069 (Bremner et al 1992) can be used to enhance the clinical effectiveness of treatment. Certain specific agents can be used to alter sensitiser metabolism and improve treatment effects. For example, an iron chelator such as desferrioxamine can be used in ALA PDT. This further slows the conversion of protoporphyrin IX to haem, leaving more PPIX within tissue to be available for light activation. The other prerequisite for PDT in addition to light and sensitiser is of course oxygen. It would be tempting to think that the use of hyperbaric

oxygen would enhance the treatment effect. While this may be possible, experience from radiotherapy trials has not been encouraging.

12.6.5 Other Combinations

One of the first applications of the porphyrins was as a radiosensitiser. Currently this has not been shown to be of great clinical value, but it remains an interesting area for research.

12.7 Summary and Conclusion

PDT is a clinical reality now. There are some established indications and indeed some licensed indications for its use. This however is merely the beginning. A great deal of preclinical and clinical work still needs to be done to improve and optimise this therapy if it is to become a useful clinical treatment. Currently, this is focused on techniques to match the PDT effect to the full extent of the tumour, including a safety margin in the knowledge that normal tissues can tolerate treatment. This requires refinement of dosimetry and real time treatment monitoring. Ultimately, these parameters need to be calculated and incorporated into the treatment in a semi automated way. If PDT is to find wide application, it must be easily learned and carried out, or it will suffer the same fate as brachytherapy in Europe – that is to say it will decline until it is only carried out in a small number of institutions, even though it is of proven value.

It may be that future applications will also be in totally different spheres of medicine such as the treatment of benign disease like lymphangioma, neurofibroma and haemangioma, or in microbiology. We would do well to remember that the original observation of the PDT effect was the result of sunlight falling on a paramoecial culture. Multiple resistant staphylococcus aureus is currently reaching epidemic levels in our intensive care units and hardly a year goes by without the discovery of another “superbug,” so we may yet need to revisit this important indication for PDT.

Plus ça change, plus c'est la même chose!

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Appendix

Appendix 1

UICC TNM Classification of Malignant Tumours (4th edition, 2nd revision), published by P Hermanek and LH Sobin and published by Springer Verlag, Berlin, 1992

TNM Criteria for Oral Squamous Carcinoma and Minor Salivary Gland Carcinoma

Primary Tumour Size (T)

- TX Primary tumour cannot be assessed
- T0 No evidence of primary tumour
- Tis Carcinoma *in situ*
- T1 Tumour less than 2cm in greatest dimension
- T2 Tumour 2-4cm in greatest dimension
- T3 Tumour more than 4cm in greatest dimension
- T4 Tumour with extension to involve adjacent structures (eg through cortical bone, into deep (extrinsic) muscles of the tongue, into maxillary sinus or skin)

Regional Lymph Node Involvement (N)

- NX Regional lymph nodes cannot be assessed
- N0 No regional lymph node metastasis
- N1 Metastasis in one ipsilateral node less than 3cm in greatest dimension
- N2
 - N2a Metastasis in a single ipsilateral lymph node 3-6cm in greatest dimension
 - N2b Metastasis in multiple ipsilateral lymph nodes, none more than 6cm in greatest dimension
 - N2c Metastasis in bilateral or contralateral lymph nodes, none more than 6cm in greatest dimension
- N3 Metastasis in a lymph node more than 6cm in greatest dimension

Stage Grouping

Stage	TNM Classification		
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1	M0
Stage IV	Any T4 lesion		
	Any N2 or N3 lesion		
	Any M1 lesion		

Appendix 2

ICD Codes in the Head and Neck WHO Classification of Diseases

- 140 Malignant neoplasm of lip
- 141 Malignant neoplasm of tongue
- 142 Malignant neoplasm of major salivary glands
- 143 Malignant neoplasm of gum
- 144 Malignant neoplasm of floor of mouth
- 145 Malignant neoplasm of other and unspecified parts of mouth
- 146 Malignant neoplasm of oropharynx
- 147 Malignant neoplasm of nasopharynx
- 148 Malignant neoplasm of hypopharynx
- 149 Malignant neoplasm of other and ill-defined sites within the lip, oral cavity and pharynx

Appendix 3

Suppliers of equipment:

Diomed Ltd, Cambridge Research Park; Ely Road; Cambridge CB5 95E; UK

Applied Optronics Corporation, 111 Corporate Boulevard, Building J, South Plainfield, New Jersey, USA 0870

Laserscope, 3052 Orchard Drive, San Jose, California, USA 95134-2011

Medlight SA, Ch des Larges Pieces, CH-1024 Ecublens, Switzerland

Pioneer Optics Company, 1000 Old Country Circle, Windsor Locks, Connecticut CT 06096, USA

CeramOptec GmbH, Siemensstrasse 44, D-53121, Bonn, Germany

Suppliers of Drugs:

Foscan®: Scotia Pharmaceuticals, Scotia House, Castle Business Park, Stirling, Scotland FK9 4TZ, United Kingdom

ALA: DUSA Pharmaceuticals Inc, USA

Photofrin®: QLT Phototherapeutics, Vancouver, Canada

Appendix 4

Publications arising from this thesis:

Fan KF, Hopper C, Speight PM, Buonaccorsi GA, MacRobert AJ, Bown SG. Photodynamic therapy using 5-aminolaevulinic acid for premalignant and malignant lesions of the oral cavity. *Cancer* 1996 Oct 1;78:1374-1383.

Fan KF, Hopper C, Speight PM, Buonaccorsi GA, Bown SG. Photodynamic therapy using mTHPC for malignant disease in the oral cavity. *Int J Cancer* 1997 Sep 26;73(1):25-32.

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